VOLUME LXXXVIII NUMBER 4

THE

BOTANICAL GAZETTE

December 1929

STRUCTURE OF LARGE SOMATIC CHROMOSOMES

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(WITH PLATES XXII–XXIV AND ONE FIGURE)

Introduction

A number of years ago the writer published two papers (41, 42) dealing respectively with the large somatic chromosomes of *Vicia faba* and *Tradescantia virginiana*. According to the descriptions given therein, the chromosome, apparently homogeneous in the anaphase, became visibly differentiated during the telophase into more chromatic and less chromatic constituents by a process which has previously been called "alveolation." The less chromatic constituent mingled with other fluids to form the karyolymph, not necessarily persisting as a portion of the individualized chromosome throughout the mitotic cycle. The more chromatic constituent of each chromosome constituted a portion of the metabolic reticulum, assumed the form of a slender thread in the ensuing prophase, underwent a longitudinal division, and then thickened and shortened to become the double metaphase chromosome. This chromatic constituent was held to persist throughout the nuclear cycle, assuming the form of a slender thread (chromonema) only during the early prophase, when its longitudinal division appeared to take place.

Since that time certain other observers, notably MARTENS (23, 24), KAUFMANN (12–14), and KUWADA (16), have furnished cogent evidence in support of the view that in such chromosomes the two constituents mentioned are morphologically distinct and often

visible, even in the metaphase and anaphase, and that the appearance of an alveolation in the telophase is due to a gradual decrease in the chromaticity of the less chromatic constituent, which renders increasingly visible a structure already present. The opinions of these observers differ as to the history of the less chromatic constituent, but the more chromatic constituent is thought to maintain the form of a more or less continuous chromonema throughout the mitotic cycle. KAUFMANN, moreover, following certain writers on animal chromosomes, contends that there are *two* chromonemata in the chromosome at all stages, owing to the early period at which the division of the more chromatic constituent is accomplished. In view of certain points to be brought out later, special mention should also be made of the statement of MARTENS that in *Paris quadrifolia* and *Listera ovata* the chromonema is single in the anaphase and the early prophase, and becomes doubled in the middle and late prophase, not by actual splitting, but by a less regular reapportionment of its substance to the two sides of the chromosome.

This general view, that the chromosome in its most condensed phases is essentially a matrix inclosing one or two chromonemata, has received strong support in the results of studies on chromosomes in the meiotic period. Especially fruitful have been the use of neutral violet extra on living chromosomes by KUWADA and SUGIMOTO (19) and KUWADA (17), the boiling water method of SAKAMURA (36, 37), the refinements of older methods by TAYLOR (46) and KAUFMANN (13), and the studies on the relation of hydrogen-ion concentration to visibility of chromosome structure by KUWADA and SAKAMURA (18) and SAKAMURA (36).

The theoretical value of such a conception of chromosome structure, which was developed especially by BONNEVIE (1–3) and VEJDOVSKÝ (47), has long been obvious in the light of other direct evidence that many chromosomes have characteristic morphological lengthwise differentiations, and the more indirect but scarcely less convincing evidence that they may have a lengthwise differentiation in function also.

In resuming this study of somatic chromosomes after an interval of several years, the writer has made use of certain special methods, to be described later, together with some of the methods used in the

earlier investigations. The account of the chromosome cycle based on the results so far obtained is not intended to be complete, for a considerable number of significant points are still very obscure. The results are nevertheless sufficient to warrant a reinterpretation of the former observations, as well as a somewhat definite statement regarding the general course of events in the chromosome cycle as now conceived.[1] The data now at hand are in full harmony with the view that the more chromatic constituent of the somatic chromosome maintains the form of a chromonema, double in some cases at least, throughout the mitotic cycle, and is accompanied by the less chromatic constituent as a matrix at most if not all stages. The telophasic transformation does not involve an actual "alveolation" of the chromosome; hence this term should be abandoned.

Materials and methods

The present investigation was carried out on chromosomes in the root tips of *Trillium grandiflorum* (Michx.) Salisb., *Allium cepa* L., *Podophyllum peltatum* L., *Vicia faba* L., and *Tradescantia virginiana* L. Fixations were made in the usual manner with a number of fluids, including those of Benda, Flemming, Merkel, and Bouin. Particular attention was given to the action of Benda's fluid in order to obtain an adequate basis of comparison with the former results on *Vicia* (**41**), which had shown the superiority of this fluid for the fixation of the desired details. Moreover, the direct observations of MARTENS (**24–27**) on the action of this fluid under the microscope have indicated that it fixes the nuclei without producing any fundamental alteration in their visible structure. The most useful stain was iron-alum haematoxylin, both long and short procedures being employed. Among a variety of counterstains tried, eosin and chromotrope appeared to give the best differentiation of the chromosome matrix from the karyolymph.

Much of the uncertainty regarding the minute structure of chromosomes has been due to the difficulty of obtaining a suitable differentiation of the more chromatic and less chromatic constituents during late prophase, metaphase, and anaphase, when both

[1] The substance of this paper was originally presented before the General Section of the Botanical Society of America at its meeting in New York on December 28, 1928.

constituents tend to stain deeply with haematoxylin or safranin. In the present study it was attempted to increase the difference in chromaticity between the two constituents, and thereby to gain a clearer picture of chromosome structure, by bringing about an incipient solution, digestion, or other alteration of the chromosome before fixation and staining, the thought being that the relative chromaticity of the constituents might be changed before their structural arrangement was noticeably disturbed. Such a procedure was suggested by the observations of OES (**30, 31**) on the effects of temperature, alcohol, toluol, and other reagents on the process of autolysis in plant and animal cells; and by those of NĚMEC (**28, 29**) on the solubility of chromosomes in hot water, with and without previous treatment with alcohol.[2] The "granular remains of mitosis" and the vague structures in treated chromosomes reported by these workers (NĚMEC **29,** pl. IV) prompted testing the possibility of obtaining more definite and instructive results with such treatments.

More than twenty different procedures were tried, chiefly on the root tips of *Allium*. These included the use of warm and hot water, ethyl alcohol of various strengths and temperatures, sodium phosphate, potassium nitrate, potassium hydroxide, sodium chloride, carbolic acid, corrosive sublimate, and other reagents. With very few exceptions these treatments yielded nothing of immediate value. Two of them, designated "method twenty" and "method four," proved to be more useful, and may therefore be described further.

METHOD TWENTY.—Fresh roots were placed in 12 per cent ethyl alcohol at 40° C. and allowed to remain for various lengths of time, after which they were fixed in Benda's fluid over night. They were imbedded in paraffin, sectioned, and stained in the usual manner. Anaphase chromosomes treated thus with warm alcohol for 30 minutes show only a slight swelling when compared with the controls, but they exhibit a decidedly clearer structure in properly stained preparations. After as long a treatment as four hours their appearance is much the same. Evidently the alteration which renders the chromosome matrix relatively less chromatic occurs within the first few minutes, after which any further change proceeds very slowly.

METHOD FOUR.—Fresh roots were placed in 95 per cent ethyl al-

[2] See also the general accounts of ZACHARIAS (**48**) and PRATJE (**33**).

cohol for 15 minutes and then transferred to distilled water at 90° C. for a few seconds or minutes. This treatment was followed by fixation in Benda's solution, as in method twenty. Roots which had been in the hot water for 15–60 seconds exhibited chromosome structure almost identical with that shown after the other method, whereas after four or five minutes the chromosomes appeared translucent with no clear structure. This method was suggested by NĚMEC's (28) observation that previous treatment with alcohol retards the rate of solution in hot water. Without such preliminary treatment, water at any temperature above 60° C. swells and dissolves the chromosome too quickly to be of much service. The statements of NĚMEC and OES are confirmed that "resting" nuclei are far more resistant to the drastic action of hot water than are the metaphase or anaphase chromosomes.

This application of such methods can scarcely be regarded as more than a preliminary survey, since it is probable that any of them which show promise could be considerably refined by giving special attention to such factors as, for example, the conditions under which the roots are grown and the pH value of all the solutions employed. As will be pointed out in the description of the anaphase, it seems abundantly evident that the structure described is not produced, but only rendered more visible, by the special treatments, since it frequently appears with marked clearness in chromosomes not so treated.

Preliminary statement

A brief summary and diagram of the interpretation of the somatic chromosome cycle are first presented, in order to facilitate detailed description and to furnish a convenient basis for any future discussion. The scheme here outlined should not be regarded as one to which all the species dealt with conform rigidly in all particulars; especially should it not be applied without further investigation to chromosomes in tissues of other types. It is an interpretation which seems to represent a somewhat close approach to the state of affairs in all the large somatic chromosomes here studied, but only future researches can show in what particulars it is inadequate, or how it must be modified to conform to the conditions found in other chromosomes.

In the anaphase the chromosome is made up of a matrix and a more chromatic constituent, the latter having the form of a crooked thread or chromonema (text fig. 1). The thread often appears double, that is, there are two chromonemata, which may either remain very close together (upper part of chromosome) or separate rather widely (lower part), both now and in the other phases of mitosis. At the end of the anaphase (tassement polaire) the chromosome shortens.

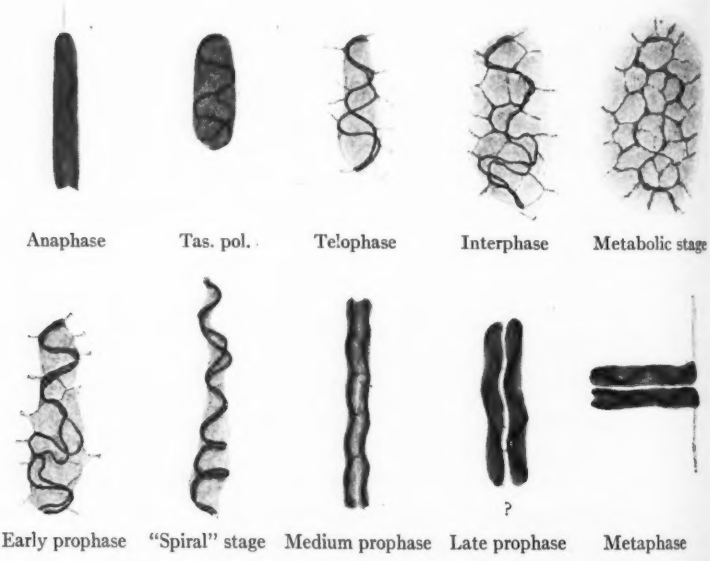

FIG. 1.—Diagram of successive alterations undergone by large chromosome during course of one mitotic cycle: chromosome matrix shown in gray and chromonemata in black; exact stage of prophase at which each of the two chromonemata becomes doubled is undetermined.

In the telophase the chromaticity of the matrix decreases, leaving the chromonemata more plainly visible. These are more contorted than in the anaphase, owing to the shortening of the chromosome as a whole. They become connected with the chromonemata of the neighboring chromosomes by anastomoses to form a reticulum.

The telophasic alterations continue into the interphase, when the individual chromonemata can still be traced in favorable prep-

arations. Rapidly multiplying nuclei may pass into the next prophase from this stage. When the divisions are less rapid the telophasic alterations are carried further, the anastomoses and the main portions of the chromonemata becoming more and more alike, so that the latter are no longer discernible in the fine-meshed metabolic reticulum. The chromatic matter is not resolved into visible granules. Meanwhile the matrix substance of the several chromosomes becomes entirely achromatic and apparently continues. The precise relation of this substance to the karyolymph in which the reticulum is imbedded is undetermined.

In the early prophase the reticulum is resolved into separate tracts representing the chromosomes, in each of which the two chromonemata become increasingly visible as the anastomoses disappear. The matrix of each chromosome becomes distinguishable from the surrounding karyolymph. The chromonemata become increasingly distinct and regular, giving the aspect characteristic of the spiral stage.

In the middle portion of the prophase the chromonemata straighten, thicken, and diverge somewhat as they take up positions in the two borders of the flattening matrix. The matrix becomes more chromatic, and probably begins its division.

In the late prophase the chromosome matrix is shorter, thicker, and still more chromatic, and its division is completed. The chromonemata are again contorted, but owing in part to the chromaticity of the matrix, their exact structure is obscure. In view of their evident duality in each half-chromosome in the metaphase so soon to follow, together with certain very suggestive appearances in the late prophase, it is highly probable that each chromonema undergoes division some time during the latter portion of the prophase, as indicated provisionally in the diagram. The two chromonemata thus formed are to separate finally in the second succeeding anaphase.

In the metaphase each half-chromosome (daughter chromosome) is a matrix containing two chromonemata, so that the entire metaphase chromosome is double with respect to the matrix and quadruple with respect to the more chromatic constituent. The intimacy of association of the two chromonemata in each half-chromosome varies greatly, making the duality obvious in some cases but not evident in others.

Observations

The following description does not present a continuous account of the alterations undergone throughout the mitotic cycle by the chromosome in a given species; rather it takes up the phases of mitosis in turn, describing whatever chromosomes have yielded information, regardless of species. There are still gaps or points of uncertainty in the known history of the chromosome in each species, but the measure of general agreement between all the species warrants the adoption of the provisional scheme presented in the foregoing pages, and lends weight to the opinion that actual differences are chiefly those of degree.

The anaphase is considered first because of the obvious advantage of beginning with the stage at which the chromosome is most clearly evident as an individual. Furthermore, the special technical methods were employed with particular reference to their effect upon metaphase and anaphase chromosomes, in which the structure is ordinarily obscured by the chromaticity of the matrix.

For purposes of comparison it should be noted that all of the figures in the plates are drawn at the same magnification, with the exception of figs. 1, 8, 17, 18, 20, 26, 39, and 49, which show only half as much enlargement. These smaller scale drawings show the general appearance of the nuclei and facilitate identification of the various phases.

Anaphase

In most ordinary preparations the anaphase chromosomes appear as uniformly black or gray rods (fig. 1, *Vicia*). That such chromosomes are in some degree double is strongly suggested by the notch and light median line occasionally seen at the distal end (fig. 2), as well as by the moniliform outline frequently reported (Kaufmann 13). The most extreme condition so far observed is in *Trillium* (fig. 3), in which the anaphasic doubleness may be as conspicuous after fixation in Benda's fluid as one expects it to be in the late prophase or the metaphase after some other fixations. These chromosomes are somewhat flattened, and have deeply chromatic borders separated by a more readily destaining median region. The light region is plainly not the familiar median line often produced by refraction.

An apparently different type of structure is revealed in anaphase

chromosomes by special methods, and frequently by ordinary methods as well. In fig. 4 are shown two chromosomes and a portion of a third in a root tip of *Vicia* fixed in Benda's solution and stained with iron-alum haematoxylin (short procedure). Here the chromatic constituent has the form of a very evident zigzag or spirally coiled chromonema running more or less continuously throughout the length of the chromosome. At the upper end of each large chromosome there is a suggestion of duality, while in the small portion of a chromosome there appear to be two chromonemata, although this is not a particularly clear case. Such internal chromatic tracts in chromosomes fixed in Flemming's weaker solution were figured in an earlier paper on *Vicia* (**41**), but the proper significance was not assigned to them.

The same type of internal structure appears clearly in chromosomes of *Allium* prepared by method twenty. Except for the decreasing length and increasing thickness of the chromosome, the appearance is the same in early anaphase (fig. 5), medium anaphase (fig. 6), and the tassement polaire stage (fig. 7). The matrix has a somewhat even outline, and is distinctly delimited from the surrounding fluid. In some regions the more chromatic element exists as an apparently single zigzag or spiral thread, whereas in other regions there are as clearly two of these. The probable key to this situation is found in cases such as that in fig. 6. In *a* there is but one evident chromonema; in *b* the chromonema is double at the distal (lower) end; in *c* there are two widely separated chromonemata, as in fig. 5*bc*. Later a similar series of aspects will be reported for the telophase and the prophase.

A useful hypothesis covering these phenomena in such chromosomes is that the chromonematic element is actually double at all stages, and that its halves vary greatly in their spatial arrangement, owing in part to the conditions of growth, methods of fixation, and other unknown local conditions. Moreover, much variation in appearance depends upon the angle from which the associated chromonemata are viewed. Consequently, the chromosome may appear to have a single chromonema, a double chromonema, or two chromonemata. To what extent fixation may effect a divergence of the threads, or their closer approximation as suggested by KAUFMANN,

must remain for the future to determine. That such spiral structures are artifacts of fixation is scarcely a plausible view, now that KUWADA and SUGIMOTO (**19**) have demonstrated them in fresh meiotic chromosomes with neutral violet extra, and since SAKAMURA (**36, 37**) has dissolved away the matrix, leaving the spirals intact.[3]

TELOPHASE

In the comparatively young telophase nucleus (fig. 8, *Vicia*) the individual chromosomes stand out conspicuously, owing to their straightness and the transparency of the non-chromonematic substances. Their exact structure, however, is not so easily distinguished as would appear from that fact. The chromonemata undergo considerable distortion, especially at places where anastomoses connect them with one another; also, the chromosomes may soon begin to curve somewhat, and the spaces between them gradually become less evident.

The middle chromosome in fig. 8 is shown on a larger scale in fig. 9. Although the precise arrangement of the chromatic substance is uncertain at a number of points, it is plain that it continues to be in the chromonematic form seen in earlier stages. In view of what has preceded, it is best interpreted as a pair of chromonemata. In the left-hand chromosomes in figs. 10 and 11 the two chromonemata are more obvious, since they tend to run more nearly parallel in certain regions. In fig. 12 (*Tradescantia*) they appear to be widely separated above and more closely associated below, although this apparent difference may be due entirely to the angle of view. It is to be doubted whether the two chromonemata in such chromosomes as that shown in fig. 12 are so interlaced or intertwined that they could not be moved apart laterally without becoming entangled. This point will be mentioned again in the account of the prophase.

In figs. 13 (*Tradescantia*) and 14 (*Allium*), which are drawn from nuclei a little more advanced than that of fig. 12, there appears to be but one chromonema. Such an aspect is undoubtedly due in some

[3] While this paper was in press, the visibility of the chromonema in the chromosomes of living *Tradescantia* sporocytes in Ringer's solution was reported by MARTENS (Bull. Acad. Roy. Belg. Cl. Sci., Ser. V **15**: 160–169. 1929).

cases to the fact that two chromonemata lie in the same line of vision, or to imperfect fixation or staining. I am of the opinion, however, that in the telophase, as in the anaphase, the two chromonemata vary considerably in the intimacy of their association, so that they may at times lie in contact and form what appears to be a single thread. It is even possible that this tends more strongly to occur as the telophase advances, either naturally or because of imposed conditions. This is suggested by the fact that in these preparations the appearance in the succeeding interphase is so often one of singleness (figs. 15, 16).

Those who have studied such nuclei are aware of the basis for the interpretation of the conditions shown in figs. 8–14 as results of alveolation, especially when it is borne in mind that in very young telophase nuclei (between the stages of figs. 7 and 8) the chromosome matrix is more chromatic and tends to obscure the structure actually present in most ordinary preparations. In such preparations the first indication of a loss of chromaticity in the matrix is the appearance of translucent spots, and as the process continues these spots become the spaces between and around the chromonemata. Thus they may appear to originate in very irregular positions within each chromosome, as stated in earlier papers (**41, 42**). The situation is well illustrated in KAUFMANN'S figures of meiotic chromosomes (**13,** figs. 56–63). Now that we know the chromonemata to be present even in the preceding anaphase, it is evident that the structure observed within telophase chromosomes is not actually produced at this time by alveolation or any other process, but is simply rendered visible through a reduction in the chromaticity of one of the constituents of the chromosome. The only conspicuous new elements are the anastomoses, which join the chromonemata of neighboring chromosomes to form the interphasic reticulum.

Brief reference may be made here to the views of certain earlier writers with respect to telophasic doubleness. It was the opinion of LUNDEGÅRDH (**20, 21**), FRASER and SNELL (**9**), DIGBY (**6, 7**), FRASER (**8**), and others that median alveoles appear in telophase chromosomes and develop into a more or less complete longitudinal split, which persists through the succeeding interphase and prophase. Accordingly the chromosomes of the telophase were figured as some-

what ladder-like structures, the two side pieces corresponding to the future daughter chromosomes. This particular interpretation of the telophase was opposed in my earlier papers, since it was found (1) that the alveoles appeared not only in a median position but along the periphery as well; (2) that the chromosome at this time was still a cylinder and not a flattened ladder; and (3) that the side pieces of the ladder-like telophase chromosome did not develop directly into the longitudinal halves of the succeeding middle prophase chromosome, but gave rise in the very early prophase to an apparently single slender thread incorporating also the cross pieces, this thread then splitting to give the duality of the middle prophase. It now appears that the foregoing investigators were correct in interpreting some (although not all) of the telophasic aspects they observed as chromosome doubleness, although they shared my error in supposing the telophasic alteration to consist in an actual alveolation. Furthermore, they were wrong in believing the splitting to take place in the telophase as the result of such a process. Later it will be shown that splitting is in all probability prophasic, as I (**41, 42**), KUWADA (**15**), OVERTON (**32**), and others have contended, although it does not occur in the manner hitherto supposed.

MARTENS (**23, 24**) also holds that the partisans of the theory of splitting in the telophase were correct in their general observation of chromatic duality at that stage, but were wrong in their interpretation. For him, however, the duality they observed does not represent an actual definitive splitting of the chromosome as they contended, but results from the temporary accumulation of the chromatic material along two lines corresponding to the two edges of a troughlike structure formed by this material at earlier stages. This interpretation he supports by the statement that an unsplit chromonema is present in the early stages of the succeeding prophase. The "trough" conception does not seem applicable to the appearances presented by my preparations; moreover, it will be pointed out later that the prophasic chromonema is double from its earliest stages. Hence it can only be concluded that, although the investigators referred to were wrong in their general interpretation of the telophase, the duality they observed at that stage was at least in some cases (cases in which the chromonemata were widely separated, as in my

fig. 12) actually that which develops into the doubleness of the succeeding middle prophase.

Interphase

As the telophase advances and passes into the interphase, the nucleus increases in size and the reticulum formed by the anastomosed chromonemata tends to become more nearly uniform throughout. The individual chromosomes accordingly stand out less clearly, while the analysis of their structure becomes increasingly difficult. A small piece of an exceptionally clear interphasic reticulum of *Vicia* is shown in fig. 15. Here the contorted chromonemata appear to be single and rather thick, as if the two in each chromosome had become very closely associated, as suggested in a previous paragraph. Somewhat the same appearance is presented by a treated interphase nucleus in *Allium* (fig. 16). Other cases are better interpreted as two more loosely associated chromonemata, the aspect of each chromosomal tract being more like fig. 12. Hence it seems that the several conditions present at earlier stages owing to a variation in the mutual behavior of the chromonemata may be continued into the interphase.

The foregoing interpretation of the development of the interphasic reticulum from the chromonemata of the anaphase is in its essential features similar to that of Kaufmann (13). The most noticeable difference is with respect to the duality visible in the later stages: I do not find in my preparations such regularly intertwined chromonemata throughout the telophase nucleus as Kaufmann shows in his figs. 24, 27, and 29. With respect to the interphase, my fig. 15 and his fig. 32 are in closer agreement except for the degree of angularity of the meshes of the reticulum. In his figure many of the zigzag threads appear to be single, although duality is visible in certain places, as I have also observed. It is my intention to study more intensively the telophasic alterations in the chromosomes of *Trillium*, for in view of their well developed duality in the anaphase it would seem that they should yield valuable evidence as to the constitution of the interphase nucleus.

One of the most puzzling problems pertaining to the telophase and interphase is that of the fate of the chromosome matrix, and hence the constitution of the achromatic substance in which the

reticulum is imbedded. A discussion of the various possibilities has been presented elsewhere (SHARP 43) and need not be repeated here. In a young telophase nucleus of the stage shown in fig. 8 the chromosome matrix appears to be distinct from the fluid which occupies the spaces between the chromosomes, and which is probably entering the now enlarging nucleus. Later on, however, when the reticulum becomes more uniform and the matrix less chromatic this distinction is not evident, and I have inclined to the opinion that the chromosome matrix and the fluid entering the nucleus during the telophase intermingle to form the transparent karyolymph of the interphase and metabolic stage. This is also the view of KAUFMANN; MARTENS, on the contrary, believes the two substances to remain distinct. As yet no published figures can be regarded as decisive on this point.

The general appearance of an advanced interphase (of *Tradescantia*) is shown in fig. 17. There are no obvious boundaries between the chromosomes at this stage, although in properly oriented nuclei one may still distinguish chromonematic tracts representing the chromosomes. The extent to which the telophasic transformation may be carried varies with the interval between successive mitoses. In any given case it may be impossible to be sure that the nucleus is to enter the next prophase without further alteration toward a metabolic condition, but the frequency with which the various stages occur in some sections, and comparisons of their arrangements in certain cases, indicate that the turning point may be reached before the stage of fig. 17, that is, while the chromonematic elements of the individual chromosomes are still distinguishable. In rapidly multiplying nuclei, conditions more advanced than that of fig. 17 are comparatively rare, but as the interval between mitoses becomes longer, completely developed reticula are found.

METABOLIC STAGE

A fully formed metabolic reticulum from the same section as the interphase of fig. 17 is shown in fig. 18. The structure of this nucleus is similar to that of the preceding stage in all respects save the greater tenuity of the chromatic strands and the smaller size of the meshes in the reticulum. Accordingly, tracts representing individual chromosomes can no longer be identified. The network is uniform through-

out, and lies in the transparent karyolymph which affords no evidence of the presence of two physically distinct constituents. When viewed under low magnification or when insufficiently stained, the nucleus may seem to be filled with discrete granules, but careful observation of good preparations always reveals the presence of strands connecting the granules. In other words, the supposed granules are only those regions of the chromatic strands which are thicker or are being viewed endwise.

The validity of interpretations of nuclear structure based on fixed material alone may rightly be questioned. The usual statement of those who have studied living cells under carefully controlled conditions is that most metabolic nuclei appear faintly granular or entirely homogeneous except for the nucleolus. SCHAEDE (**39**) found the living nuclei of young *Allium* and *Vicia* root cells to be homogeneous in appearance, not showing the reticula reported by LUNDEGÅRDH (**20, 21**) until they become moribund. In older root cells and in the stamen hair of *Tradescantia* he observed a granular appearance. His general conclusion is that the chromatic matter in metabolic nuclei has the form of discrete granules suspended in the karyolymph, these two constituents not being visible in some nuclei because of their similarity in refractive index. The reticulum he regards as an artifact due to degenerative changes in living cells or to fixation in ordinary preparations.

MARTENS (**24–27**), working with the roots of *Listera* and other plants, the leaf of *Elodea*, the stamen hair of *Tradescantia*, and especially the plumose stigmas of *Arrhenatherum elatius* and other grasses, showed that under carefully controlled conditions of observation unharmed nuclei may reveal the presence of reticula, and not merely discrete granules. Furthermore, he watched the action of Benda's and Bouin's fluids upon such nuclei under the microscope, and found that the reticular structure visible before fixation remained essentially unchanged, although there might be a slight swelling of the filaments, especially at the nodes of the reticulum, and an increase in their visibility through alterations in refractive index. The same details appeared in material fixed for longer periods and stained with iron-alum haematoxylin in the usual way. SCARTH (**38**) also reported "an irregular framework of soft gel" in the living nuclei

of *Tradescantia*, a finely granular aspect in *Elodea*, and apparent homogeneity in *Spirogyra* and the fruit of *Symphoricarpos*. The visibility of the reticulum in these cases is said to vary inversely with the pH value and more or less directly with the viscosity of the nucleus as a whole.

The opinion which is best justified by a comparison of all reported observations is that the chromatic matter in metabolic nuclei of the type here dealt with exists in the form of a reticulum composed of anastomosed chromonemata. The actual appearance of the living nucleus (whether homogeneous, granular, or reticulate) depends upon the refractive indices of the chromatic matter and the karyolymph. Increasing divergence of these indices through natural or artificial causes brings into view first the nodes ("granules") of the reticulum and then the finer connecting strands, in much the same manner that progressively deeper staining does so in fixed material. If we regard as valid SCHAEDE's argument that the appearance of granules in the *Tradescantia* stamen hair nucleus as opposed to the homogeneity of the root nuclei he saw is due to different refractive properties in different unharmed nuclei, it would seem to be equally applicable to the connecting filaments observed by MARTENS in nuclei of the same kind; in fact, as MARTENS points out, SCHAEDE's own photomicrograph (pl. V, fig. 6*f*) shows crooked filaments and not only granules.

It is possible, of course, that there are other types of nucleus to which the foregoing interpretation will not apply. GROSS (11) reports brownian movement of apparently isolated granules in the nuclei of the salivary gland of *Limnaea*, and SHIWAGO (44) describes filaments which seem to be unconnected with one another in frog leucocytes. The observations of CHAMBERS (4) on the prophase stages in living spermatocytes of *Dissosteira* afford strong support to the view that fixation does not produce the structures in question, but only renders them more visible. That actual coagulation artifacts do occur at times goes without saying.

PROPHASE

EARLY PROPHASE.—For convenience of treatment the whole prophase may be subdivided into three portions. What is here called

the early prophase is characterized by the resolution of the reticulum into its individual chromosomes, in each of which the chromatic constituent appears as two associated chromonemata. The changes undergone by the chromosomes are in many respects the reverse of those in the telophase, at least so far as appearances are concerned; if it were not for the early disappearance of the anastomoses, the resemblance between telophase and prophase nuclei would be even closer.

As the prophase is initiated, the first visible change consists in the attenuation of the anastomoses along certain lines in the reticulum (fig. 19). This alteration may begin in a metabolic reticulum (fig. 18) or in the relatively coarser interphase stage (figs. 15, 17). The successive cells in a row in the root do not often show a regular succession of mitotic phases; this, together with the resemblance of late telophase and early prophase nuclei, often renders it impossible to be certain regarding the immediate past of a given nucleus in these stages. Somewhat later the prophase nucleus is easily distinguishable from the earlier telophase nucleus (compare figs. 20 and 26 with fig. 8) by its larger size and more evenly rounded contour, the relative scarcity of anastomoses, and the absence of a similar sister nucleus in the same or adjacent sections. In the very early prophase nucleus, in which the reticulum is just beginning to break up, the chromatic tracts (chromosomes) tend to be broader and the anastomoses more tenuous than in the early interphase (compare figs. 19 and 15, drawn from nuclei in the same preparation). As the actual turning point between successive mitoses is approached, all these criteria naturally become less useful, since at this point the differences vanish. However, they do permit one to follow the telophase far enough along, and to trace the prophase far enough backward, to make it practically certain that in regions of the root where the nuclei are multiplying rapidly, and where very advanced interphases are relatively rare, the turning point may be reached before the constituent chromonemata become indistinguishable. Accordingly, the stage shown in fig. 19 may be considered to follow that of fig. 15 directly. The probability that this interpretation is valid is very high, although more convincing proof should be sought in some tissue showing a regularly arranged series of mitotic phases.

The exact structure of the chromatic tract representing the chromosome at the beginning of the prophase is difficult to determine. In some cases there appear to be two chromonemata more or less separated, but in the majority of clear examples observed the chromatic thread appears to be for the most part single. It is partly for this reason that these examples are more clearly analyzable. In the lower part of the left-hand chromosome of fig. 19 a single zigzag seems to be coming into prominence; that the lighter strands represent a second chromonema is doubtful. In view of the tendency of the two chromonemata to associate closely in the telophase and the interphase, and their obvious closeness in the prophase to follow, it seems more than likely that such apparently single zigzags as that in fig. 19 consist of two parallel elements.

As the anastomoses disappear and the chromatic tracts become isolated, the nucleus has the appearance shown in fig. 20 (*Podophyllum*). Under moderate magnification, as in this figure, most of the chromatic matter in each tract appears to form a single crooked thread with duality suggested at some points. This is the "apparently single slender thread incorporating also the cross pieces" of the telophase chromosome referred to earlier in the paper. Under high magnification the duality can be seen plainly. Several chromosomes or portions of chromosomes from nuclei of *Vicia* in this stage are illustrated in figs. 21–23. In the lower portions of the chromosomes in fig. 21 the chromonemata lie closely parallel, while in the upper portion of fig. 21*a* they seem to be widely separated. The latter condition is rather plain in the upper portion of fig. 22*b*. Some chromosomes, such as those illustrated in fig. 23, are scarcely interpretable in most regions: they are much like the "alveolized" prophase chromosomes figured in my earlier papers. The fact that the chromosomes of figs. 22 and 23 are from the same nucleus indicates that the "alveolized" examples are only poorer pictures of the chromonematic structure shown to better advantage in the other figures. A similar variation is found in *Podophyllum:* in fig. 24 the general structure appears to be very irregular and the chromonema single at most points, but in another chromosome from the same preparation (fig. 25) the chromonema is clearly double at the end where the matrix has been destained to just the right degree. All of these cases

point to the conclusion that two chromonemata are present in each chromosome from the inception of the prophase, a point of the greatest importance with reference to any interpretation of the chromosome cycle.

As a result of the disappearance of anastomoses and the increasing regularity in the arrangement of the chromatic matter, the conspicuous "spiral stage" of the early prophase is reached (fig. 26, *Podophyllum*). The ease with which this stage is recognized is due in part to the tendency of the two associated chromonemata in each chromosome to lie very close together, whatever may have been their previous position. Figs. 27–38 show portions of chromosomes in this stage more highly magnified. Here, as in the still earlier prophase, the chromatic thread may appear single or double, depending upon fixation and other circumstances. In *Vicia*, for example, it appears single in fig. 27 (Flemming fixation) but plainly double in fig. 37 (Benda). In *Trillium* it may be apparently single in most regions (fig. 29) or obviously double (fig. 30) in the same preparation (Benda). In fig. 31 (*Podophyllum*) are shown three chromosomes in the same nucleus: in the uppermost the successive coils of the spiral thread are very close together; in the middle chromosome the thread has for some reason straightened out in a curious manner, but shows no duality; in the lowermost the thread is unmistakably double in its middle region, where the extended condition makes it certain that the double appearance cannot be due simply to a closeness of successive coils. Duality of the thread is further shown in *Tradescantia* (figs. 32–35), although the appearance varies here as in the foregoing examples.

A particularly clear example is shown in fig. 37 (*Vicia*). Here the two chromonemata have thickened a little more than is usual before the mid-prophasic straightening, which makes it possible to follow the course of the threads with less uncertainty. In this portion of the chromosome they form a strikingly regular spiral. Moreover, careful examination of the points at which the two threads cross each other strongly suggests that they are not simply intertwined, but that their relative position is such as would permit them to move apart to two sides of the enveloping matrix without becoming entangled. That they should be able to do this is suggested further by the fact

that they have been brought into the spiral form by the shortening of the matrix at another stage of the mitotic cycle, as will soon be shown. KUWADA (**17**) has considered this aspect of the problem, and has discussed it instructively with the aid of wire models. He points out that if two wires forming a common spiral are twisted once around each other for every turn of the spiral, but in the direction opposite thereto, they will come apart without entangling. In fig. 37 it appears very much as if the chromonemata might diverge, one toward and one away from the observer, without becoming entangled; it is scarcely possible, however, to be altogether certain of this. Later on in the prophase they do become well separated and parallel, but to what extent this may involve twisting and other modes of rearrangement is not known.

Attention may be directed again to the variation in the appearance of the chromatic matter, especially in different preparations and lots of material, and to the idea that this is due in considerable measure to influences causing approach or divergence of the two associated chromonemata during growth and fixation. It is well known that the distinctness of half-chromosomes in the metaphase varies in this way, and it therefore seems not unlikely that corresponding effects are produced at other stages also.

This variable appearance in the slender chromatic threads of the prophase (singleness in one region and doubleness in another) has usually been interpreted as an actual splitting at this stage, particularly when the threads have undergone some straightening before thickening (fig. 38). Such was the interpretation given in the paper on *Vicia* (**41**), fig. 10 of that paper showing about the same stage as fig. 38 of the present one. That such an interpretation is valid for the chromosomes in question can no longer be maintained, in view of the demonstrated duality at earlier stages. In many preparations an apparently single crooked thread emerges in the early prophase and becomes double later, but in other preparations of the same lot of material the early thread also shows the duality.

This point is of further interest in connection with the problem of the meiotic prophase. To cite briefly but one recent investigation, SZAKIEN (**45**) reports that in *Osmunda* the thread arising from each chromosomal tract in the presynaptic stages is single, and that no

duality is visible in such threads until well after their parasynaptic association has been accomplished. Although phenomena in somatic nuclei must be used with some reserve in explaining the meiotic changes, the present observations tend to support the suspicions of many cytologists that the chromosomes in meiosis are in some measure constitutionally double some time before their synaptic association begins.

The chromosome matrix is not shown clearly in most of my figures of the early prophase, largely because they were drawn from preparations destained to show the chromonemata as clearly as possible. Moreover, in the earliest stages it has only the slightest affinity for the haematoxylin stain. In darker or counterstained preparations the matrix can be seen, and appears to be distinct from the karyolymph (figs. 23, 25, 27); but later on, when the slender chromatic threads straighten (fig. 38) it becomes difficult or impossible to identify it in many regions. I incline to the view of MARTENS that the matrix exists throughout this stage as a thin layer about the chromatic threads and as small accumulations held in their curves. The alternative view is that of KAUFMANN, who believes the matrix substance to differentiate anew in the later prophase, only the chromonemata preserving the identity of the chromosomes through the interphase and early prophase. As already emphasized, the whole subject of the less chromatic component of the chromosome and its relation to the karyolymph deserves further intensive study.

MIDDLE PROPHASE.—The change characterizing the middle portion of the prophase is the straightening and thickening of the chromonemata; this gives the familiar mid-prophase aspect shown in fig. 39 (*Podophyllum*). In what measure this change involves an actual elongation of the chromosome as a whole is undetermined. In fig. 38 the straightening threads are still slender, which would seem to necessitate a considerable increase in length; whereas, in fig. 37 a noticeable amount of thickening has occurred while the threads are still contorted. The aspect varies to some degree with fixation, but it nevertheless seems likely that the time relation of straightening and thickening is not always precisely the same. Were these changes to proceed at the proper relative rate, there would be no elongation of the chromosome as a whole.

Although the chromonemata in any given region of a chromosome have become much straighter, they remain more or less twisted about each other, and together follow an irregular course through the nucleus (fig. 39). The free ends of chromosomes not at either surface of the section show that there is no continuous spireme. The associated chromonemata tend to diverge more than in the earlier stages of the prophase, and occupy the edges of the now flattening and broadening matrix. A rather extreme case is shown in fig. 40. In the light preparations from which this and a number of the accompanying figures were drawn, the matrix is colorless, but the delicate strands connecting the chromonemata can be distinguished. In fig. 41 is a small portion of a chromosome in which the two chromonemata at first appear to be twisted, but in which careful focusing seems to show the same thread above the other at all crossing points. Fig. 42 illustrates the lumpy appearance so often presented by the threads at this stage. Nothing new can be contributed here regarding the origin and significance of these lumps, or chromomeres.

The prophasic changes so far described are of special interest in connection with the view of MARTENS (**23, 24**) that the chromonematic element of the chromosome does not actually split, but that its substance undergoes a less regular reapportionment or redistribution ("répartition bilatérale chromatique") to the two borders of the flattening matrix. According to this view, the two chromatic strands in the middle prophase (fig. 42) and later are the result of such a process initiated in a single thread (chromonema) present in the earlier prophase. Since KAUFMANN and I have now shown that there are two chromonemata in the chromosome (that is, that the chromatic thread is not single but double) from the beginning of the prophase, it follows that I (**41**) was in error in attributing the doubleness of the middle prophase to a splitting in the early prophase, and also that the interpretation of MARTENS is equally untenable. As the two chromonemata gradually straighten and take up positions at opposite edges of the matrix, they with their connecting strands often present aspects similar to those figured by MARTENS; but the two threads supposed by him to be formed in the middle and late prophase are actually present much earlier, so that another explanation of their origin must be found.

The probability that the morphological division of the chromatic substance is initiated in the middle prophase (in each of the two chromonemata present from an earlier stage) will be discussed later.

LATE PROPHASE.—The late prophase is characterized by a thickening and shortening of the chromosome with the resultant contortion of the chromonemata and division of the matrix. There is also evidence that the doubleness of each chromonema becomes visible at this time; if so, this constitutes the most important feature of all.

The effect upon the appearance of the chromosome produced by its shortening and thickening, and by the contortion of the chromonemata, is illustrated in figs. 43–47, only some of which show the matrix. These five figures represent respectively chromosomes of *Allium* prepared by method twenty, *Vicia* fixed in Flemming's strong fluid, *Podophyllum* fixed in Benda's fluid, *Tradescantia* fixed in Flemming's fluid, and *Trillium* fixed in Benda's fluid. All are from preparations stained with iron-alum haematoxylin; that of *Trillium* was counterstained with eosin and shows the matrix more plainly. Just as it is difficult to say in what measure the straightening of the chromonemata in the middle prophase is correlated with an elongation of the chromosome as a whole, so in the late prophase one cannot be sure of the degree of interdependence of chromosome shortening and chromonema contortion. That the two changes are causally related, however, can scarcely be doubted. Whether the chromonemata themselves undergo any actual changes in length with their alteration in form during the mitotic cycle is not known; it is even conceivable that they maintain a nearly constant length, and vary in appearance from stage to stage according to the shapes assumed by the matrix in which they are confined.

As the prophase advances the chromosome matrix gradually increases in chromaticity, so that the internal structure of the chromosome tends to become obscured. As in the anaphase, the chromaticity may be decreased by special treatment; moreover, it is sometimes sufficiently less in material not so treated to reveal the structure rather clearly. In fig. 46 the two chromonemata are rather well separated in the matrix. They appear at first glance to be twisted; but one lies above the other, and if this chromosome could be viewed

from the side the appearance would probably approach that of fig. 47*a*, in which the chromosome ribbon lies flat in the section.

The exact stage at which the matrix becomes completely divided is not easily determined; observations on this point are insufficient to warrant very positive statements. In the very late prophase chromosomes in fig. 47 the matrix appears to be continuous from one side to the other; whereas, in the chromosome in fig. 48, which was observed in a nucleus apparently of the same age, the matrix has divided. Doubtless the inception of the division in some regions may occur at a considerably earlier stage: this is suggested by the unusually wide divergence of the two chromonemata sometimes observed (fig. 40). How much variation there may be in this respect is not known.

At the end of the prophase, therefore, the two daughter chromosomes which are to separate in the following anaphase are defined, their matrices having become distinct only recently, but their chromonematic elements having been distinct for a much longer period. Before proceeding with a description of subsequent phases, special consideration must be given to the chromonemata.

It has already been stated that two chromonemata are present in the anaphase chromosome, and it will soon be pointed out that this chromatic duality is evident in each half of the double metaphase chromosome, at least in some cases. It would therefore seem that a subdivision of each of the two chromonemata seen in the early prophase must occur some time before the metaphase. Direct and convincing evidence on this point is not easily obtained. In the late prophase chromosomes shown in figs. 44, 45, and 47 there are suggestions of doubleness at certain points (indicated by arrows), but the real significance of these aspects cannot be evaluated safely at present. It is for this reason that a mark of interrogation appears at the late prophase stage in the diagram in the text. This is essentially the point to which KAUFMANN (13) carried his study of the chromonemata in *Tradescantia pilosa:* his figs. 47, 48, and 50 show strong hints of chromatic duality in each half of the late prophase chromosome. On theoretical grounds we could expect the division to be initiated while the chromonemata are relatively slender, as in the middle

prophase, even though it might not attain visibility until the chromosome had thickened somewhat in the late prophase. It is intended to investigate this matter further, especially in the large chromosomes of *Trillium*.

The process by which the chromonema becomes doubled longitudinally is as obscure as ever, perhaps more so. In view of the actual division of other protoplasmic units, such as cells and plastids, it is natural to assume that the chromonema also is doubled by an actual fission. To what degree this is the case, that is, to what extent and in what manner the molecular and colloidal constituents of the chromonema are involved in division, can only be conjectured. It is conceivable that a process similar to that supposed by MARTENS to occur in a single thread in the prophase may take place in each of the halves of this actually double thread in the preceding prophase, but at present there is no reason to regard this as very probable. Since such great theoretical importance attaches to this point in the mitotic cycle, it is to be hoped that a way will be found to obtain more decisive observational data.

If the interpretation of the course of somatic nuclear division embodied in the diagram is correct, the chromosome as a whole does not become fully divided until the fission of the matrix late in the prophase of the mitosis in which the resulting daughter chromosomes are to separate; whereas, its more chromatic constituent (chromonema) has been divided since the prophase of the preceding mitosis. If, as some observers maintain, the matrix does not remain distinct from that of the other chromosomes and from the karyolymph during the interphase, it would be necessary to say that the double "chromosome" (chromonema) of the early prophase becomes surrounded by a new individual mass of matrix substance, making a single "chromosome" (matrix) which is divided in the late prophase. The conception of the autonomy and genetic continuity of the chromosome would accordingly apply only to the chromonematic element. To what extent the less chromatic constituent may participate in determining the morphological and functional peculiarities of the chromosome is an interesting and important question.

METAPHASE

An illustration of the stage transitional from the late prophase to the metaphase is introduced (fig. 49, *Podophyllum*), not for any evidence it affords regarding chromosome structure, but because of its bearing on the theory of spindle origin set forth by DEVISÉ (5) for microsporocytes and by ROBYNS (**34, 35**) for somatic cells. In the root tip nuclei of *Hyacinthus* and *Vicia*, ROBYNS found that as the prophase comes to a close the nuclear membrane contracts about the chromosomes, leaving behind two polar caps of karyolymph which become the spindle cones. Hence the spindle is composed primarily of karyolymph, although this may be modified in some way through interaction with non-nuclear constituents.

In *Podophyllum* essentially the same series of changes has been observed. The nucleus shown in fig. 49, however, is exceptional in that its membrane has disappeared with little or no shrinkage. The contour of the nucleus remains as it was in the preceding late prophase stage, except for a slight extension at the lower pole. The karyolymph shows the striations characteristic of fixed spindle substance. The insertion points of the chromosomes have assumed their metaphasic positions in the equatorial plane, and show the small projections which suggest the beginning of anaphasic movement. The condition illustrated in this figure seems to leave no escape from the view that the spindle in such cells is composed primarily of karyolymph.

With regard to the structure of the chromosomes in the metaphase, the most instructive figures so far observed are those in *Trillium grandiflorum*, the plant in which GRÉGOIRE and WYGAERTS (**10**) described "alveolar" chromosomes at this stage. In fig. 50 there appears to be but one thick chromonema in each half-chromosome, just as one is often led to believe for other stages (figs. 4, 14, 29, etc.). In fig. 51, however, each half shows two well separated chromonemata; this stage may be considered to follow that of fig. 47. Still more striking are the conditions illustrated in figs. 52–54: here the chromonemata are somewhat thicker and more nearly parallel, which gives each half-chromosome the aspect ordinarily presented by a whole chromosome in late prophase (cf. fig. 45). A further indication of duality in each half-chromosome is frequently found at the inser-

tion point. In fig. 55 a terminally inserted chromosome of *Trillium* shows four peglike projections, two on each half. Similarly, subterminal insertion points may show four small bridges across a relatively achromatic region (fig. 56). Only a few observations of this nature have been made; hence no decided statement regarding the significance of such appearances is warranted. A further special study of the chromonemata at constrictions and insertion points would probably yield information of much value.

The structure illustrated in figs. 50–54 may be seen not only in material specially treated, but occasionally also in material prepared in the usual manner. These five figures are all from material of the latter kind, the fixation being made in Benda's fluid; and with the exception of figs. 52 and 53, all are from different roots. A full explanation of the variation in these metaphase chromosomes is not yet at hand, but I have made use of the provisional hypothesis that the two chromonemata show different degrees of approximation or separation under different conditions of growth and fixation, and that the processes concerned in their doubling vary in some measure with respect to the phases of the mitotic cycle. A certain amount of variation of this kind occurs in nuclei of one preparation, as is indicated for stages other than the metaphase; and there is even more diversity when one compares different lots of material of the same species, and especially of different species. The most advanced stage of doubling in metaphase half-chromosomes observed is in *Trillium*.

In view of what has frequently been claimed regarding the dependence of the division of other organic units upon their growth and absolute mass, I have been led to inquire whether the precocity in the division of the chromonemata in the chromosomes here described may not be related to their unusually large size. Suppose, for example, that the morphological division of the chromonema occurs when its growing mass reaches a certain critical value, or when the multiplying molecular or colloidal units of which it is composed reach a certain number, this value or number not necessarily being precisely the same for all cases. In a large chromosome we might expect this critical point to be reached at an earlier stage of the mitotic cycle than in a small chromosome, inducing the doubling of the chromonema further in advance of the time at which its halves are

to separate. If this were so, doubling might be detected at various mitotic phases in different tissues or in different species; or, it is possible that some condition peculiar to the prophase may determine the rearrangement of materials which results in a visible doubling of the chromonema only in that phase, even if the mass of substance or the number of constituent units has reached the critical value at an earlier stage. In the latter case the visible division of the chromonema would always be prophasic, although in large chromosomes it might appear one full mitotic cycle earlier than in small chromosomes. Accordingly, small chromosomes may be chromatically double in the metaphase, but not quadruple as in the large chromosomes of *Trillium*. Whatever conception of chromosome structure one may derive from the study of large chromosomes, however, there is always open the possibility that small chromosomes may have that same structure but do not reveal it to the eye because their constituent elements are smaller or more compactly arranged. This touches such significant questions as that of the relative number and arrangement of genes in chromosomes of different size, which makes it all the more desirable to obtain some evaluation of the foregoing speculations.

Conclusions

1. The large somatic chromosomes of the five plants studied consist of two principal morphological constituents. These differ decidedly in their affinity for stains during late telophase, interphase, metabolic stage, and early prophase, but not so greatly during late prophase, metaphase, anaphase, and early telophase.

2. The more chromatic constituent of each chromosome persists throughout the mitotic cycle in the form of two chromonemata. The less chromatic constituent forms a matrix in which the chromonemata lie during most of the cycle. It is uncertain whether it remains distinct from the matrix substance of the other chromosomes and from the karyolymph during the interphase, or loses its identity between mitoses.

3. The series of alterations undergone by the two principal constituents of the chromosome through the successive phases of the mitotic cycle are summarized with the aid of a diagram in the preliminary statement near the beginning of the paper. From that

statement it follows that the respective matrices of two sister chromosomes separating in the anaphase are defined by a division at the end of the immediately preceding prophase; whereas, their respective chromonematic constituents are defined by a division in the second preceding prophase.

4. It is suggested that if the division of nuclear elements is related in a definite way to their growth and mass, the stage in the mitotic cycle at which the chromonema becomes longitudinally doubled may not be precisely the same in all nuclei. Hence the interpretation given here for large chromosomes, in which the chromonema divides one complete mitotic cycle in advance of the matrix, should not be extended to small chromosomes without a special study of appropriate material.

5. Intensive studies with more refined methods should be made of the following uncertain points: (1) the precise stage at which the division of the chromonema begins in chromosomes of all sizes; (2) the manner in which this division may involve any smaller elements; (3) the morphology of the chromonema with respect to chromomeres; (4) the arrangement and behavior of chromonemata with respect to constrictions, satellites, and insertion points; (5) the influence of cultural conditions and fixation upon the spatial arrangement of the chromonemata in a chromosome; (6) possible alterations in the length of the chromonema during the mitotic cycle; (7) the measure in which the matrix of each chromosome retains its identity through the interphase; (8) the origin of the extra-chromosomal karyolymph so abundant in the late prophase, and its relation to the matrix substance of the chromosomes; (9) the effect of a larger variety of conditions and reagents upon the relative chromaticity of the chromosomal constituents; (10) the precise rôle of the nucleolus; (11) the physical and chemical characteristics of the chromosomal constituents at different stages; (12) the relation of the chromonemata to the matrix through meiosis, with special reference to the mechanism of crossing-over.

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[*Accepted for publication February 2, 1929*]

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EXPLANATION OF PLATES XXII–XXIV

All figures were drawn with the aid of an Abbe camera lucida, the mirror distance being increased in most cases to give greater enlargement. The drawings have been reduced one-half in reproduction, and show magnifications as follows: figs. 1, 8, 20, 26, 39 and 49, ×1750; figs. 17, 18, ×1900; all other figures, ×3500. The following lenses were used for critical examination: Zeiss apochromatic objective, 2 mm. N.A. 1.40; compensating oculars 6, 8, 12, and 18; immersed achromatic condenser, N.A. 1.40. Special care was taken to obtain critical illumination. Figs. 17 and 18 are reprinted from the paper of 1920 (42).

PLATE XXII

FIG. 1.—*Vicia:* general appearance of long and short chromosomes in anaphase.

FIG. 2.—*Vicia:* anaphase chromosomes, showing indications of doubleness at distal end (Benda).

FIG. 3.—*Trillium:* portions of anaphase chromosomes, showing doubleness (Benda).

FIG. 4.—*Vicia:* anaphase chromosomes with chromonemata (Benda).

FIG. 5.—*Allium:* anaphase chromosomes with chromonemata (method 20).

FIG. 6.—*Allium:* slightly later than fig. 5; note duality in *b* (method 20).

FIG. 7.—*Allium:* tassement polaire; two chromonemata in upper chromosome, apparently only one in lower (method 20).

FIG. 8.—*Vicia:* general aspect of medium telophase (Benda).

FIG. 9.—*Vicia:* middle chromosome of fig. 8.

FIGS. 10, 11.—*Vicia:* telophase chromosomes, showing two chromonemata in some regions (Benda).

FIG. 12.—*Tradescantia:* telophase chromosome with two chromonemata (Flemming).

FIG. 13.—*Tradescantia:* same, with apparently one chromonema (Flemming).

FIG. 14.—*Allium:* telophase, with apparently one chromonema (method 4).

FIG. 15.—*Vicia:* interphase, with anastomosed chromonemata apparently single (Benda).

FIG. 16.—*Allium:* portion of interphase nucleus prepared by method 4.

FIG. 17.—*Tradescantia:* general aspect of interphase nucleus (Flemming).

FIG. 18.—*Tradescantia:* general aspect of metabolic stage (Flemming).

PLATE XXIII

FIGS. 19–38: early prophase.

FIG. 19.—*Vicia:* portions of two isolating chromosomes (Benda).

FIG. 20.—*Podophyllum:* general aspect of early prophase nucleus (Benda).

FIG. 21.—*Vicia:* duality visible at some points (Benda).

FIG. 22.—*Vicia:* chromonemata well separated in places (Benda).

FIG. 23.—*Vicia:* matrix stained; interpretation doubtful (Benda).

FIG. 24.—*Podophyllum:* chromonema irregular and apparently single in many regions (Benda).

FIG. 25.—*Podophyllum:* end of chromosome in which chromonema is clearly double in most destained portion, apparently single in middle portion, and invisible in darkest portion (Benda).

FIG. 26.—*Podophyllum:* general aspect of nucleus in "spiral stage" (Benda).

FIG. 27.—*Vicia:* spiral chromonema apparently single (Flemming).

FIG. 28.—*Vicia:* same (Benda).

FIG. 29.—*Trillium:* spiral chromonemata appearing double only in certain regions (Benda).

FIG. 30.—*Trillium:* same stage as fig. 29, but with doubleness showing clearly (Benda).

FIG. 31.—*Podophyllum:* interpretation of uppermost chromosome doubtful; middle chromosome curiously extended; lowermost chromosome showing doubleness clearly (Benda).

FIGS. 32, 33.—*Tradescantia:* doubleness apparent at some points (Flemming).

FIGS. 34, 35.—*Tradescantia:* doubleness evident almost throughout (Flemming).

FIGS. 36, 37.—*Vicia:* specially clear examples of doubleness in early prophasic chromonemata (Benda).

FIG. 38.—*Vicia:* double chromonemata tending to straighten while still very slender (Benda).

PLATE XXIV

FIG. 39.—*Podophyllum:* general aspect of nucleus in middle prophase (Benda).

FIGS. 40–42.—*Vicia:* chromosomes in middle prophase; note wide divergence of straightened chromonemata in fig. 40 and chromomeres in fig. 42 (Benda).

FIG. 43.—*Allium:* chromonemata becoming contorted as chromosomes shorten in later prophase (method 20).

FIG. 44.—*Vicia:* late prophase; chromonemata contorted and showing suggestions of possible subdivision at points indicated by arrows (Benda).

FIG. 45.—*Podophyllum:* late prophase chromosome (Benda).

FIG. 46.—*Tradescantia:* fragment of late prophase chromosome showing two chromonemata (Flemming).

FIG. 47.—*Trillium:* portions of late prophase chromosomes, showing undivided matrix with two chromonemata, in each of which duality is suggested at certain points (Benda).

FIG. 48.—*Trillium:* very late prophase; matrix divided (Benda).

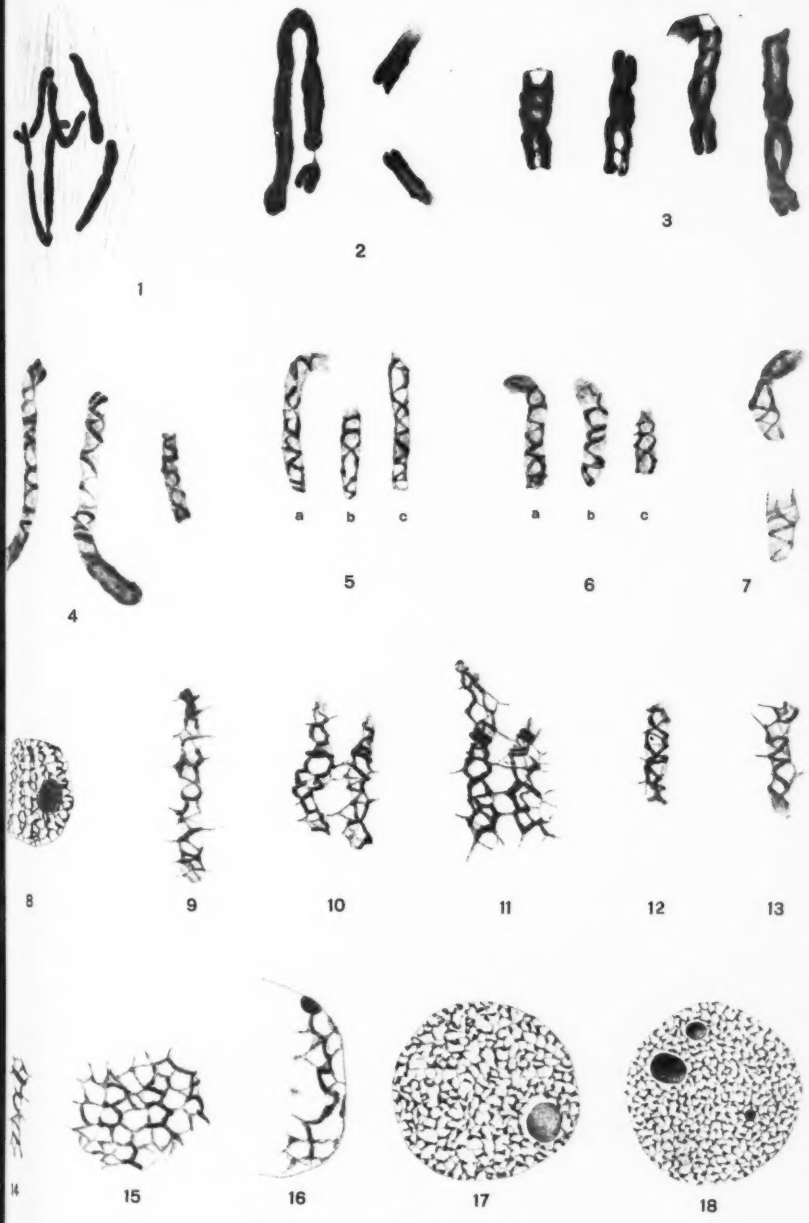
FIG. 49.—*Podophyllum:* transition from late prophase to metaphase, an unusual aspect; karyolymph forming spindle while prophasic contour of nucleus is retained; insertion points of chromosomes arranging in equatorial plane (Benda).

FIG. 50.—*Trillium:* two double metaphase chromosomes, with chromatic substance forming apparently single but heavy chromonema in each half (Benda).

FIG. 51.—*Trillium:* portion of metaphase chromosome, with two well separated chromonemata in each half (Benda).

FIGS. 52–54.—*Trillium:* metaphase chromosomes with well developed chromatic duality in each half (Benda).

FIGS. 55, 56.—*Trillium:* terminal and subterminal insertion points of metaphase chromosomes, showing indications of duality in each half (Benda).



SHARP on CHROMOSOMES

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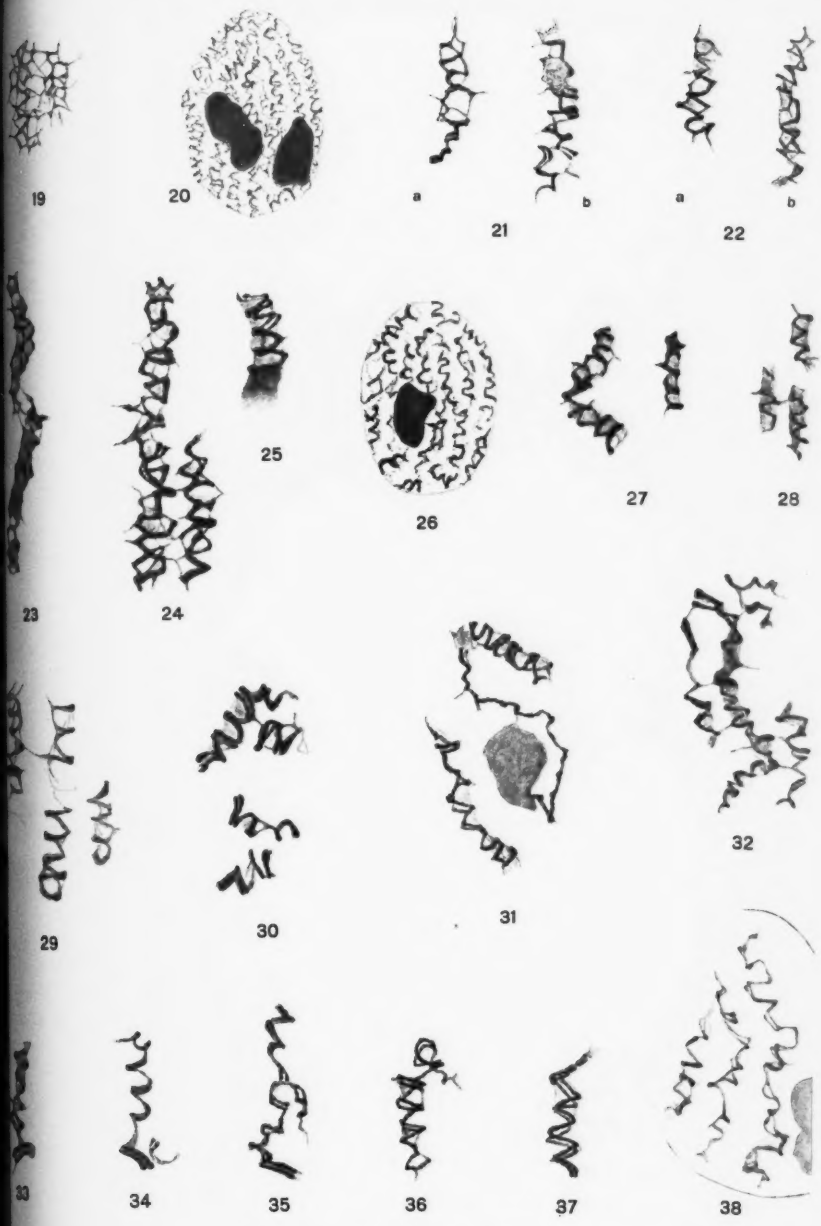

SHARP on CHROMOSOMES

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SHARP on CHROMOSOMES

CYTOLOGICAL STUDIES IN THE BETULACEAE

II. CORYLUS AND ALNUS[1]

ROBERT H. WOODWORTH

(WITH FIFTY FIGURES)

The materials and methods used in this study are described in a previous paper (WOODWORTH 19).

CORYLUS (TOURN.) L.

There is at hand no evidence which would indicate polyploidy in this genus. Ten species, four varieties, and three known hybrids have been examined, and all show the haploid number of chromosomes to be fourteen. This condition is interesting in view of the fact that the group is so closely related to the highly polyploid genus *Betula*.

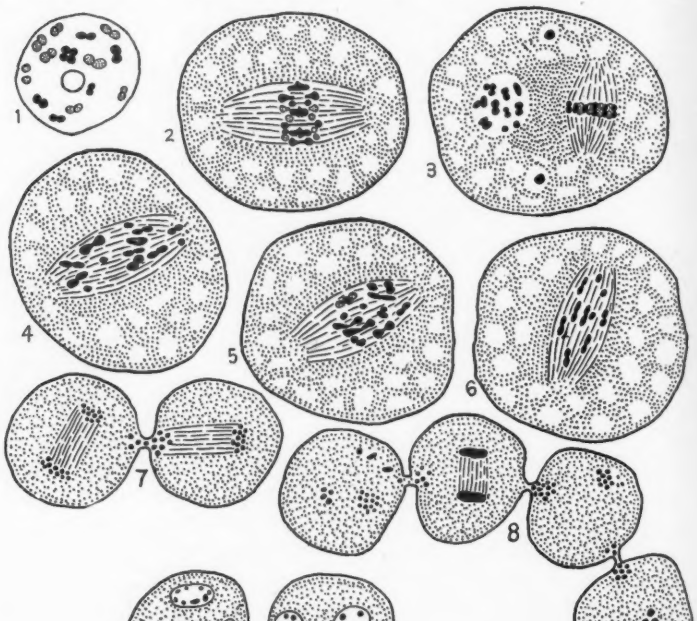
In species of *Betula* the size of the mother cell is in general proportional to the size of the chromosome complement. In *Corylus* the mother cells are as large or larger than those of *B. lutea*, which has forty-two chromosomes and the largest P.M.C. of any species of *Betula* examined. Also the chromosomes of *Corylus* are by far the smallest seen in the Betulaceae, which means that the karyoplasmic ratio (WILSON 15) in *Corylus* is several times smaller than in *Betula*. It may be worth while to note, therefore, that while this ratio is definite throughout the polyploid species of *Betula*, it is not at all constant in a comparison of the two genera.

Several times the cytoplasm has been noted to consist of large dense spheres, up to one-fifth the diameter of the cell in size.

Apparently natural hybrids are easily formed among the species. REHDER (12) has described *C. avellana*×*colurna*, *C. tibetica*×*avellana*, and *C. chinensis*×*avellana*. There is a *C. americana* × *pontica* in the Arnold Arboretum. GOESCHKE (7), in his economic treatment of the hazel nuts, presents a "Pomologische Beschreibung der Haselnüsse" in which he describes and illustrates eighty-seven sorts of *Corylus* plants which bear different types of nuts. WINKLER

[1] Contribution from the Laboratories of Plant Morphology, Harvard University.

(18), monographing the Betulaceae, recognized in *Corylus* eight species, fifteen varieties, and two hybrids. Many of the horti-



FIGS. 1–9.—Pollen mother cells and chromosome groups of species of *Corylus* during meiosis; ×2300 (except figs. 7–9): fig. 1, *C. heterophylla*, nucleus at diakinesis showing 14 bivalent chromosomes; fig. 2, *C. americana*, heterotypic metaphase; fig. 3, same, homeotypic metaphase showing extruded chromatin; fig. 4, *C. maxima* var. *atropurpurea*, heterotypic division; bivalent and univalent chromosomes lagging on spindle; fig. 5, *C. cornuta*, heterotypic division showing bivalents and univalents; fig. 6, *C. americana*×*pontica*, heterotypic division showing bivalents and univalents lagging on spindle; fig. 7, same, cytomyxis and chromosome migration at heterotypic anaphase (×1200); fig. 8, *C.* no. 9 of Vollertsen, cytomyxis and chromosome migration between four mother cells (×1200); fig. 9, *C. cornuta*, cytomyxis and nuclear migration (×1200).

cultural varieties, listed by GOESCHKE, may have arisen through hybridization.

The genus is characterized by more or less normal meioses,

which is to be expected in view of the constant number of chromosomes. If the group was typically heterozygous, a considerable degree of normality of the reduction division might prevail. Diakineses appear to consist of normal pairing, although it has been somewhat difficult to find examples of this stage. Sometimes a fusion of gemini has taken place. The heterotypic metaphase, in practically all the species examined, exhibits a pairing of certain of the bivalent chromosomes. Two or three pairs of the gemini unite, appearing in all stages, from a mere touching to complete resolution into one large chromosome mass.

Cytomyxis and chromatolysis also appear in most of the preparations. An interesting phase of the action of these plasma bridges is the migration of part or the whole of the chromosome complement from one mother cell into the cytoplasm of a neighboring mother cell.

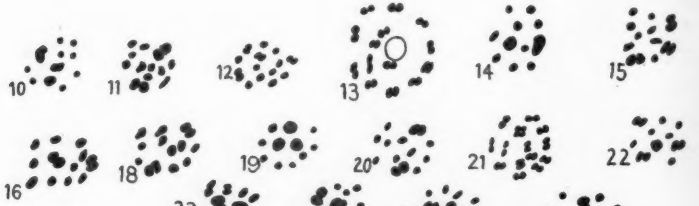
Corylus americana Walt., X-14 (meiosis somewhat abnormal).—Both diakineses and heterotypic metaphases occur with apparently eleven chromosomes, but there are always three large clumps, each of which is composed of two gemini fused (fig. 10). The chromosomes are regular in their movements during the first division (fig. 2). The homeotypic metaphase frequently shows chromosomes extruded into the cytoplasm (fig. 3). The chromosome count of the spindle in plate view clearly indicates that these bodies are chromosomal in nature, since it does not have its full complement.

Cytomyxis is frequently seen at diakinesis, heterotypic anaphase, and at interkinesis; and is often accompanied by the migration of chromosomes across the plasma bridges (fig. 7). This is one of the possible origins of an increase in chromosome number.

C. americana×*pontica*, X-14 in both parents and in this hybrid (meiosis somewhat abnormal).—During the early heterotypic spindle the chromosomes appear as bivalent and univalent, and are distributed all over the fibers before gathering at the metaphase plate (fig. 6). The heterotypic metaphase plates exhibit all stages of the coalescence of gemini. Some plates show fourteen chromosomes very distinctly, while others show all phases from the approximation of two pairs of bivalents to their complete fusion (fig. 11). The second division is normal.

Cytomyxis is present, and the pollen is mostly normal, about 2 per cent being defective. A few microcytes appear and are no doubt caused by unequal distribution of chromosomes or by chromosome loss by cytomyxis.

C. avellana L., X-14 (meiosis mostly normal).—This species produces the filbert of commerce.



FIGS. 10–31.—Fig. 10, *C. americana*, heterotypic metaphase plate, showing characteristic condition: 3 of gemini fused, forming quadrivalents, making chromosome count appear as 11; fig. 11, *C. americana*×*pontica*, heterotypic metaphase plate; fig. 12, *C. pontica*, heterotypic metaphase plate, some chromosomes already split for second division; fig. 13, *C. avellana*, diakinesis; fig. 14, same, heterotypic metaphase plate; fig. 15, *C. avellana* var. *pendula*, heterotypic metaphase plate; fig. 16, *C. colurna*, heterotypic metaphase plate; fig. 17, *C. heterophylla*, interkinesis; fig. 18, *C. heterophylla* var. *sutchuensis*, heterotypic metaphase plate; fig. 19, *C. maxima*, heterotypic metaphase plate; fig. 20, *C. maxima* var. *atropurpurea*, heterotypic metaphase plate; fig. 21, *C. cornuta*, diakinesis; fig. 22, same, heterotypic metaphase plate; fig. 23, same, diakinesis; fig. 24, same, heterotypic metaphase plate; fig. 25, *C. sieboldiana*, heterotypic metaphase plate; fig. 26, *C. sieboldiana* var. *mandshurica*, heterotypic metaphase plate; fig. 27, *C. spinescens*, diakinesis; fig. 28, same, heterotypic metaphase plate; fig. 29, *C. tibetica*, heterotypic metaphase plate; fig. 30, *C. villmorinii*, heterotypic metaphase plate; fig. 31, *C.* no. 9 of Vollertsen, heterotypic metaphase plate.

Diakinesis clearly shows fourteen gemini (fig. 13). Heterotypic metaphase plates show all conditions, from fourteen separate gemini to thirteen, twelve, and eleven gemini with one, two, and three large quadrivalent groups. One cell had nine bivalents, one large quadrivalent, and one very large hexivalent (fig. 14).

Cytomyxis noted at heterotypic metaphase. Sometimes as many as twelve small dark bodies are seen about the periphery of the cell

during the heterotypic division and interkinesis. These are not chromosomes, the full complement being on the spindle.

C. tibetica×*avellana* (see *C. spinescens* Rehd.).

C. chinensis×*avellana* (see *C. vilmorinii* Rehd.).

C. avellana var. *pendula* Goeschke,[2] X-14 (meiosis normal).—Three gemini are commonly fused at the heterotypic division (fig. 15). Cytomyxis was noted.

C. colurna L., X-14 (meiosis regular).—Two bivalent chromosomes commonly fused during heterotypic division (fig. 16).

Cytomyxis noted at interkinesis. Two P.M.C.'s were seen in close contact, being contained in but one callose sheath.

C. cornuta Marsh. (*C. rostrata* Ait.).—This species is commonly known as *C. rostrata* Ait., but the other name must be accepted because MARSHALL (9) published it in 1785, while AITON'S (7) publication was dated 1788.

X-14 (meiosis abnormal).—During meiosis some of the chromosomes are tardy in their actions on the spindle (fig. 5). Figs. 21 and 23 show the diakinesis, the former with fourteen gemini showing no fusion (one bivalent has already split for the second division), and the latter with two pairs of gemini fused, making quadrivalents.

The heterotypic metaphase plates give counts of fourteen, thirteen, and twelve, the two latter being due obviously to fusion of bivalents (figs. 22, 24). In fig. 22 one pair has resolved into one large chromosome, while three other pairs are close together. In fig. 24 two pairs have fused but their identity is still apparent.

The homeotypic metaphase plates have given counts of eight, nine, ten, twelve, and fourteen. These counts, other than fourteen, may be due to further coalescence of chromosomes or to chromosome loss by cytomyctic migration. Occasionally a chromosome was seen to be extruded into the plasma. This variation in chromosome number is clearly reflected in the size of the pollen grains, which occur as microcytes and intermediate sizes up to the full-sized grains. Five per cent of the pollen is defective.

Cytomyxis was noted frequently. Fig. 9 shows a typical example where there seems to be a migration of chromosomes from one cell into the next.

[2] This tree was labeled "natural variety."

C. heterophylla Fisch., X-14 (meiosis normal).—Fig. 1 shows diakinesis with fourteen gemini, one of which has split already for the second division. Fig. 17 illustrates the chromosome complement of one of the interkinetic nuclei.

Cytomyxis and chromatolysis occur during early meiosis.

C. *heterophylla* var. *sutchuensis* Franch., X-14 (meiosis normal).—Two pairs of gemini are consistently fused during the heterotypic division (fig. 18). All phases are seen, from mere approximation to complete resolution into one large mass. The cytoplasm shows large dense areas which are sometimes as broad as the spindle.

C. maxima Mill.,[3] X-14 (meiosis normal).—Three pairs of the gemini commonly fuse completely, so that the metaphase plate appears to have eight small chromosomes and three large ones (fig. 19). Occasionally a bivalent is tardy in its actions on the first spindle. Cytomyxis was noted. Pollen was entirely perfect.

C. maxima var. *atropurpurea* Dochnahl., X-14.—This variety is of garden origin. It accordingly shows some of the abnormal cytological characteristics which usually mark such plants.

In the early heterotypic division there are univalent chromosomes on the spindle but they do not cause later irregularities of meiosis. All the chromosomes are tardy in the early heterotypic spindle (fig. 4). There are usually two bivalents fused during the first division (fig. 20).

The pollen is characteristically good. Occasionally a few small or defective grains were noted.

C. pontica Koch., X-14 (meiosis entirely normal).—Fig. 12 shows fourteen distinct chromosomes, four of which have already split for the homeotypic division.

C. sieboldiana Blume., X-14 (meiosis normal).—Two pairs of gemini are usually fused (fig. 25). In homeotypic metaphase two mother cells were completely fused together, forming a large cell with four spindles.

C. sieboldiana var. *mandshurica* (Bl.) Schneid., X-14 (meiosis mostly normal).—In early heterotypic division chromosomes are occasionally tardy in their behavior on the spindle. Two pairs of gemini are consistently fused (fig. 26).

[3] Hybrids between this and *C. avellana* are commonly planted for the nuts.

C. tibetica Batalin., X-14 (meiosis normal).—Two gemini fused during heterotypic division (fig. 29).

×*C. spinescens* Rehd. (*C. tibetica*×*avellana*), X-14 (meiosis normal).—Both diakinesis and heterotypic metaphase plates show two gemini to be consistently fused (figs. 27, 28). Only 30 per cent of the pollen grains are full-sized. A large proportion are very small and many are intermediate in size. Five per cent of the pollen is morphologically sterile.

×*C. vilmorinii* Rehd. (*C. chinensis*×*avellana*), X-14 (meiosis normal).—Two gemini fused in heterotypic division (fig. 30). Cytomyxis was noted. Ten per cent of the pollen consists of microcytes, most of which are shriveled and without contents.

Corylus no. 9 of VOLLERTSEN's improved filbert varieties (labeled *C. vollertseni* in the Arnold Arboretum), X-14 (meiosis somewhat abnormal).—Heterotypic metaphase plates show two or three bivalents fused (fig. 31). In the early heterotypic division the chromosomes are tardy in their behavior, but in the anaphase they appear to migrate sharply to the poles. The homeotypic divisions are normal except that they frequently show one or two extruded chromosomes in the plasma.

Cytomyxis and chromatolysis are marked in this species, especially at prophase and interkinesis. In many anthers the P.M.C.'s are connected by cytoplasmic bridges, and during the early heterotypic metaphase there is a wholesale interchange of chromosomes. Fig. 8 shows a typical case of such actions at the heterotypic anaphase. The pollen is largely perfect. A few smaller grains appear.

ALNUS (TOURN.) HILL

This genus is of particular interest from the cytological viewpoint on account of the marked diversity and intermingling of specific morphological characters in *Alnus incana* and *A. rugosa*. Polyploidy is represented by two tetraploid species, *A. glutinosa* of Europe and *A. japonica* from Japan.

Cytomyxis was noted in all species studied. Kinoplasm is apparent in all of the figures.

A. crispa Pursh var. *mollis* Fernald, X-14 (meiosis normal).—Fig. 47 shows a late diakinesis with fourteen bivalent chromosomes.

A. maritima (Marsh.) Muhl., X-14 (meiosis normal).—Fig. 46 shows the heterotypic metaphase plate with fourteen chromosomes. Frequently one of the gemini is very late in separating. In no instance, however, has this been noted to persist long enough to cause non-disjunction. This species is commonly attributed to NUTTALL, but he (10) distinctly notes that MUHLENBERG is the original author.

A. incana (L.) Moench., X-14 (meiosis normal).—Fig. 43 shows the normal metaphase spindle of the first division; fig. 44 shows the anaphase of the same division. Fourteen chromosomes are clearly seen at each pole. Fig. 45 shows the two homeotypic spindles. No cells showing abnormalities of meiosis have been seen in material of this species. Small darkly stained bodies have frequently been noted about the periphery of the cell. They are minute in comparison with a chromosome, and are clearly not chromosomal in nature.

There is some question as to just where the specific lines of this species and *A. rugosa* begin and end. The material here reported has all been taken from specimens which exhibit the characters set forth in the seventh edition of GRAY'S Manual. When these characters are all present, the bark on the older parts of the shrub shows conspicuous, raised, horizontal lenticels.

A. rugosa (DuRoi) Spreng. In bulletin no. 145 of the Vermont Agricultural Experiment Station it is stated that *A. incana* and *A. rugosa* intergrade around the lake shores. In GRAY'S Manual the following statement appears under *A. rugosa:* "Many shrubs near the northern limits of this range appear intermediate between this and the last species (*A. incana*)." WINKLER (18) lists hybrids between *A. glutinosa* and *incana*, *A. glutinosa* and *rugosa*, and *A. incana* and *rugosa*. The writer has examined many stands of alder in Maine, and very frequently it was impossible to place an individual as typical *A. incana* or *A. rugosa*. Professor FERNALD has kindly shown the writer collections of *Alnus* from various parts of northeastern America which are of the *incana-rugosa* group, but which possess characters that do not allow them to be classed with either one species or the other. With our present understanding of this complex, it is possible to say of a plant one of three things: it is *A. incana*, it is *A. rugosa*, or it is of them but not one of them.

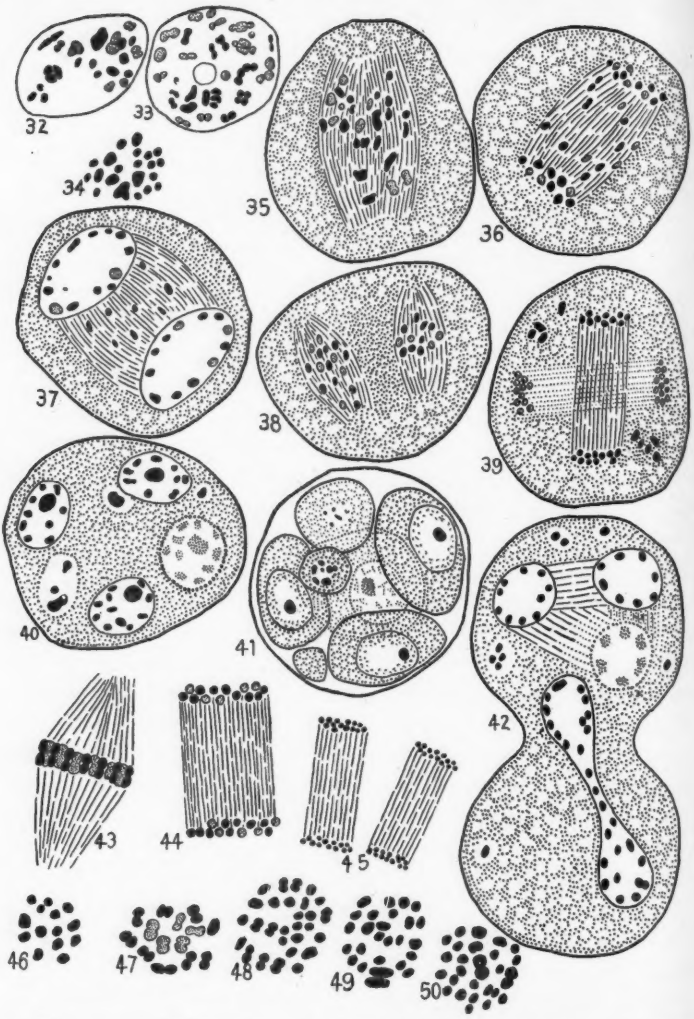
In writing of *A. rugosa*, the writer refers to the plant which grows in northern and middle New England. The opportunity to study material of *A. rugosa* from the southern United States has not yet been realized; there the species may be quite stable.

It is the usual occurrence to find extensive tracts of *A. rugosa* practically destitute of staminate catkins. Clumps of several hundreds of shrubs have been noted to possess but a few of these sterile aments which have 95 per cent of their pollen sterile. Since the plants set very large quantities of seed and there is obviously not nearly enough pollen to fertilize them all, it appears that either they are pollinated by *A. incana* or they form their seed apomictically.

Large numbers of the fertile catkins have been collected and examined cytologically from the time of the shedding of pollen in April up to July 4. In no case had the embryo sac yet been formed, and during May to July the stigmas were black and withered. No indications of pollen grains have been seen on the stigmas during these months, and in no case was the pollen tube observed in the top of the ovary. In view of the large number of preparations examined, it is reasonable to suppose that this plant forms its seeds apomictically. Further investigation has proved this point (WOODWORTH **20**). There is an ever increasing understanding that parthenogenesis is an end product of hybridization. Since there is ample reason to believe that *A. rugosa* is parthenogenetic, there is an accompanying suggestion that the plant is of hybrid origin. The cytological study of microsporogenesis, now to be described, indicates clearly that *A. rugosa* is heterozygous.

The material which is here reported on has been taken from specimens exhibiting the commonly recognized characters of *A. rugosa*. The lenticels also appear to shed some light on the differentiation from *A. incana*. These air passages on the older bark are small, inconspicuous, round, and very close together in vertical rows; in marked contrast to the prominent, horizontal lenticels of *A. incana*, although a few of this latter type are commonly scattered among those typical of *A. rugosa*.

X-probably 14 (meiosis extremely abnormal).—Fig. 33 illustrates the late prophase at the diakinetic stage. This has proved to be a difficult phase to study because of the peculiar pairing and non-



FIGS. 32–50.—Pollen mother cells of species of *Alnus* during meiosis. *A. rugosa:* figs. 32, 33, nucleus at diakinesis; fig. 34, heterotypic metaphase plate; fig. 35, early heterotypic division, no equatorial plate formed, chromosomes distributed all along spindle; fig. 36, heterotypic anaphase, many chromosomes lagging on spindle; fig. 37, heterotypic telophase, several chromosomes excluded from daughter nuclei; fig. 38, early homeotypic metaphase showing unequal distribution of chromosomes; fig. 39, homeotypic anaphase showing extruded masses of chromatin; fig. 40, polycaric (multinucleate) mother cell showing abnormal condition resulting from chromosome extrusion; fig. 41, polyspory; seven pollen grains unequal in size instead of four normal grains; small grains and many large ones degenerate; fig. 42, abnormal behavior of two mother cells. *A. incana:* fig. 43, spindle of heterotypic metaphase (note regularity); fig. 44, spindle of heterotypic anaphase; fig. 45, spindles of homeotypic anaphase; fig. 46, heterotypic metaphase plate of *A. maritima* showing 14 chromosomes; fig. 47, diakinesis of *A. crispa* var. *mollis* showing 14 gemini; fig. 48, heterotypic metaphase plate of *A. japonica* showing 28 chromosomes, some already split for second division; fig. 49, heterotypic metaphase plate of *A. glutinosa* showing 24 bivalents and 2 quadrivalents, fig. 50, heterotypic metaphase plate of same showing 28 chromosomes.

pairing habits of the chromosomes. Some of them have paired, forming gemini, and some of the univalents have already split, as if for the second division, and appear as bivalents. Fig. 32 shows the most easily interpreted diakinesis that has been seen; there are five gemini, one trivalent chromosome body, and fifteen univalents, two of which (on the right) have already split for the mitotic division. This would indicate the diploid number of chromosomes to be twenty-eight.

Fig. 35 shows the usual condition of the heterotypic metaphase. Few gemini are formed and are at the equator, many univalents being scattered all over the spindle. Sometimes, just before the bivalents are separated, the univalents migrate to the equator. Fig. 34 illustrates the metaphase plate of such a spindle. There are seven bivalents and fourteen univalents. Some two dozen metaphase plates of the first division have been counted, a most laborious and trying procedure, most frequently showing twenty-one or twenty-two chromosomes. Six or seven chromosome groups were consistently larger and were undoubtedly bivalent in nature. One count was eighteen and showed a large knot of chromatin which must have been several chromosomes fused together. This fusion of chromosomes is the normal occurrence in *Corylus*. A few counts were twenty-three and twenty-four and showed only three and two of the bivalents. These studies of diakinesis and heterotypic metaphase show the haploid chromosome complement to be probably fourteen.

Fig. 36 shows the anaphase of the first division. The bivalents have separated normally. Some of the univalents have migrated at random, in their whole condition, to the poles; while others have been halved, each half progressing to one of the poles. This causes the chromosome number to be considerably more than twenty-eight, which would of course be the normal number.

Occasionally a few of the univalents do not arrive at the poles but remain on the spindle when the nuclear membranes are formed. This condition is shown by fig. 37. These lagging chromosomes aggregate to form dwarf nuclei and later microcytes.

The regularity of the homeotypic division depends upon the number of univalent chromosomes which have not been divided during the first division. Those which did divide do not undergo division during the second division but lag on the spindle. Fig. 38

illustrates a cell at the beginning of the homeotypic division just before the metaphase. In the first division there was apparently an unequal distribution of the univalents, because one spindle has sixteen chromosomes and the other has twelve. In this case there were no univalents left out of the interkinetic nuclei. The chromosome counts that have been made during the second division have shown from ten to sixteen (most frequently thirteen) chromosomes on each spindle. This inequality is due to the abnormalities of the first division.

Fig. 39 shows the anaphase of the second division with groups of chromosomes in the cytoplasm. These later form microcytes.

Fig. 40, a polycaric mother cell, is seen to be quite abnormal when compared with the usual normal tetrad of a true species. Fig. 42 illustrates an unusual and very irregular broad cytomyctic connection between two mother cells and consequent involvement of the nuclear actions. Such a state has not been noted by the writer in any species which is undoubtedly homozygous.

Fig. 41 shows the end product of these abnormalities. Instead of the normal quartet of pollen grains, polyspory results. The chromosomes left in the cytoplasm have formed microcytes, and the large grains differ in size, due to the loss of part of their legitimate complement. All of the dwarf grains and most of the larger grains degenerate before the pollen is shed, leaving some 5 per cent of the morphologically normal, the rest being represented by shriveled exine coats containing no protoplasm.

These abnormal conditions are now recognized as the peculiarities which obtain in hybrid plants. In a previous paper (WOODWORTH 19) unmistakable evidence in this regard is presented in the studies of two natural hybrids in this same family, *Betula jackii* and *B. sandbergi*. Due to this cytological evidence, to the probability that *A. rugosa* is parthenogenetic, and to the uncertainty of specific lines in the northern parts of its range, it is concluded that *A. rugosa* is of heterozygous origin. It is probably a cross between two species, each of which has the haploid number of chromosomes of fourteen.

A. japonica Sieb. et Zucc., X-28 (meiosis normal).—This species is tetraploid. Fig. 48 shows the heterotypic metaphase plate with

twenty-eight chromosomes, some of which are already split for the second division.

A. glutinosa Gaertn., X-28 (meiosis normal).—Figs. 49 and 50 show the heterotypic metaphase plate from polar view. In this species, as occurs typically in *Corylus*, there is a union of two or three pairs of bivalent chromosomes, so that the count appears to be twenty-five or twenty-six rather than twenty-eight. Often this union is quite apparent, as in fig. 50, but sometimes it is not so apparent (fig. 49). Occasionally the union does not occur at all, and twenty-eight chromosomes are distinctly seen.

The early heterotypic spindle appears frequently in the preparations, and was commonly tripolar with the chromosomes scattered all over it. There is an occasional slight tardiness in the action of one or two bivalents during the heterotypic and homeotypic divisions; otherwise the meiosis is quite regular.

Discussion

Light is thrown from several angles on the possible origin of changes in chromosome number. In *Alnus rugosa* the unequal distribution of chromosomes to the poles of the spindles gives rise to gametes without the normal chromosome complement. This is one of the recognized characters of hybrid plants. This same plant produces gametes with less than the full chromosome complement, through a loss of some of the chromosomes into the cytoplasm and subsequent dissolution or incorporation into a small, extra nucleus.

Cytomyxis and migration of chromosomes (WOODWORTH 19) have been seen to have taken place in several of the plants under discussion. When mother cells lose a part or the whole of their chromosome group to neighboring cells, the resulting gametes are abnormal in content.

In *Corylus* all species appear to be diploid, with fourteen chromosomes as the reduced number. Throughout the group there is a pairing of one, two, or three of the bivalent chromosomes, which often makes the chromosome count appear as less than the haploid number. The chromosome groups in figs. 1–31 show the various degrees of fusion of the gemini, from an approximation to an actual resolution into one large mass. *Alnus glutinosa* showed this same coales-

cence on the part of a few of its gemini; *A. rugosa* exhibits the same thing. This fusion has been noted in no other cells than those which undergo the reduction division. Always there is a definite number of the chromatic elements which participate in this pairing. This indicates that faulty fixation does not explain the phenomenon. Many careful observers have reported this sort of mutual attraction of bivalent chromosomes.

DIGBY (5) found that in *Primula kewensis* (*P. floribunda*×*verticillata*) two of the bivalent chromosomes join together in the heterotype prophase of the P.M.C. nuclei, forming a large quadrivalent chromosome. This union is maintained until its univalent portions separate on the spindle. This does not again take place in the homeotype, nor does it occur in the E.M.C.

In a triploid *Canna*, BELLING (2) found that nine triad chromosomes are formed. They separate into two and one at the anaphase of the first division, and are distributed at random to either pole.

In *Lactuca sativa*, GATES and REES (6) noted in over 50 per cent of the P.M.C.'s a more or less complete coalescence of two or four of the bivalent chromosomes, so that only eight or seven bodies appear on the spindle, and sometimes only five.

BELLING and BLAKESLEE (3) noted in tetraploid *Datura* that during the heterotypic prophase two pairs of gemini unite, forming quadrivalents.

BLACKBURN and HARRISON (4) found two orthoploid series of species in the Salicaceae: 19, 38, 76, and 22, 44. They suggest that the series with 19 as the fundamental number may have arisen from the other series by the fusion of chromosomes.

The *Zea mays*×*Euchlaena perennis* hybrid has thirty chromosomes which at diakinesis unite in trivalents and bivalents, or remain as univalents (LONGLEY 8).

Penstemon isophyllus and *P. campanulatus* show two pairs of chromosomes united in diakinesis, so there are generally observed six smaller and one larger pair of gemini, the latter often evidently consisting of two (WINGE 17).

RANDOLPH and MCCLINTOCK (11), reporting on a triploid form of *Zea mays*, noted at diakinesis trivalents, bivalents, and univalents. DE LITARDIERE (21) derives a nineteen-chromosome species of

Senecio from a twenty-chromosome species by the permanent union of chromosomes.

If one reviews the compilations of the chromosome numbers found in plants (TISCHLER 14, WINGE 16), polyploidy is seen to occur almost universally. Polyploidy is the occurrence of chromosome complements which are multiples of a basic haploid number. In these polyploid series dysploidy is often seen to appear. Dysploidy is the occurrence of chromosome complements which are not multiples of the basic haploid number. Frequently it is apparent that the dysploid species have arisen from the polyploid species, from one of the gametes having one or two chromosomes more or less than the haploid number, due to unequal distribution, or from a fusion of bivalent chromosomes as already described. The more extensive aberrations in chromosome number may have resulted from the participation in fertilization by a microcyte formed from dwarf spindles containing aggregations of extruded chromosomes. SHARP (13) states: "Frequently the functional gametes may show a wide range of variation in their complements, while in other instances only those with certain chromosome assortments will come to maturity and act in syngamy."

In most of the illustrations of P.M.C.'s in this report, it is apparent that the cytoplasm is more dense in the region surrounding the nucleus than it is near the outer portions of the cell. This substance has been considered as distinct from general cytoplasm, and has been variously called kinoplasm, archiplasm, superior protoplasm, and ergastoplasm. In material of the Betulaceae this zone has never appeared to be felted or to have a fibrillar structure. Although several hundreds of sporophytic cells in division have been examined, not one has ever been seen to contain this denser cytoplasm surrounding the nucleus. This would indicate that the perinuclear zone is not an artifact caused by the fixative. WILSON (15) considers "all dualistic hypotheses of the protoplasm . . . . convenient as descriptive devices only." This appears to be a very sane attitude.

Summary

1. *Corylus* exhibits no polyploidy, all species and hybrids having the haploid number of fourteen chromosomes.

2. Throughout the genus there occurs a fusion of one, two, or three pairs of bivalent chromosomes, thus often causing the haploid number to appear to be less than fourteen.

3. Natural hybrids are easily formed. These plants show some of the cytological peculiarities known to be due to heterozygosis.

4. The fundamental number of chromosomes in *Alnus* is also fourteen. Diploid and tetraploid species are described.

5. *Alnus rugosa* shows marked hybrid cytological characters and is considered to be heterozygous. It is also held to form its seed apomictically.

6. *Alnus rugosa* and *A. glutinosa* exhibit fusion of bivalent chromosomes.

7. Cytomyxis and chromosome migration take place in certain species of *Corylus* and *Alnus*.

8. Dysploidy may be due to unequal chromosome distribution, chromosome extrusion, or to cytomyxis and chromosome migration.

9. A perinuclear zone occurs in the P.M.C.'s.

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[*Accepted for publication February 13, 1929*]

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DEVELOPMENT OF SPOROPHYTE OF MARCHANTIA CHENOPODA

HELEN LOUISE MCNAUGHT

(WITH TWENTY-TWO FIGURES)

Introduction

The morphology of *Marchantia polymorpha* has been the subject of numerous investigations (7, 8, 10, 15, 17, 18, 21). Among these studies, the work of DURAND (10) has been outstanding in his description of the development of the sporophyte. It has been the purpose of the study here recorded to determine whether the sporophyte of *M. chenopoda*, a delicate South American form, varies in its development from that described for *M. polymorpha*.

The material studied was found by Professor GEORGE S. BRYAN, at an elevation of 2000 feet, on the eastern slopes of the Andes Mountains on the trail to the settlement of Pozuzo, in the Province of Huanuco, Peru. Other forms of this same species, which were not included in this investigation, were found at different levels up to 11,000 feet, the size of the thalli varying directly with the increase in elevation. At the lower elevation the thalli were uniformly small, being about 1 cm. in length and 0.4 cm. in width, while those at higher elevations were more than twice that size.

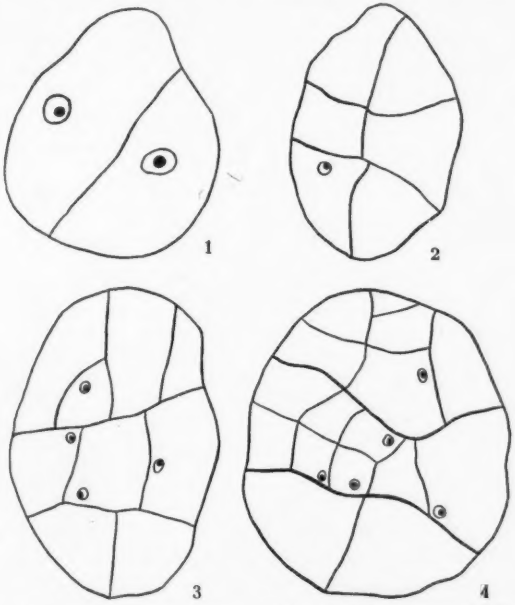
A thorough description of *Marchantia chenopoda* has been given by Professor EVANS (11), who also kindly identified the material used in this study.

The material was collected and killed at various times during the month of June, 1923. The killing fluid was made up in the following proportions: chromic acid 1 gm., glacial acetic acid 1 cc., water 400 cc.

The material was left in this fluid for about eight months, at the end of which time it was washed and run through a series of alcohol-chloroform to pure chloroform. It was then imbedded in paraffin, cut on a rotary microtome from 6 to 10 μ in thickness, and stained with safranin and licht grün.

Development of sporophyte

The first division of the zygote is, as reported for many other Marchantiales, including *Riccia* (3), *Marchantia* (3), and *Preissia* (14), somewhat obliquely transverse to the axis of the archegonium, and results in two approximately equal segments, the hypobasal and

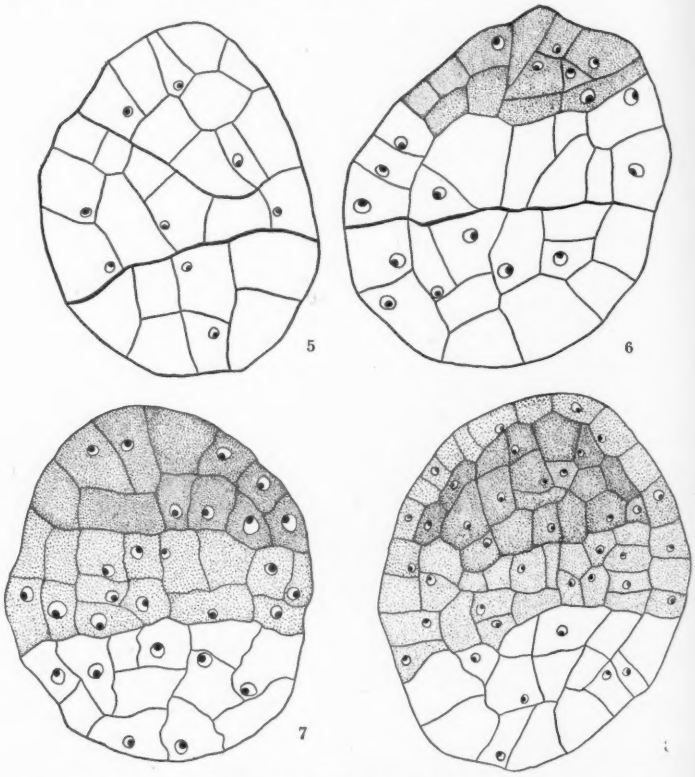


FIGS. 1–4.[1]—Fig. 1, first transverse division of zygote, very oblique; figs. 2–4, further divisions of embryo, showing conspicuous transverse divisions.

the epibasal cells. Fig. 1 shows a first division (unfortunately the only one found) which is decidedly oblique. Judging from the division walls of later embryos, it is highly probable that the first division is usually more nearly transverse. A quadrant division, such as is described for the sporophyte of *M. polymorpha* (10), has not been observed in *M. chenopoda*. Occasionally there are division walls in later embryos which might be interpreted as second division walls

[1] All figures were drawn with the aid of an Abbe camera lucida at table level, and show the following magnifications: figs. 1–8, ×1100; figs. 9–13, 18, ×700; figs. 14, 15, ×550; fig. 16, ×200; fig. 17, ×150; figs. 19–22, ×1500.

at right angles to the first wall, but this is not the most frequent occurrence. There are other divisions which are much more pronounced; namely, two primary transverse divisions which cut the



FIGS. 5–8.—Fig. 5, further division of embryo; fig. 6, early differentiation in staining reaction, showing cells of apical region somewhat darker; fig. 7, further differentiation in staining reaction, apical, middle, and basal regions very distinct; fig. 8, first appearance of differentiation in apical region between sporogenous region and cells to become capsule wall.

embryo into three distinct parts (figs. 2–5). In the following account these regions will be designated as the apical, middle, and basal regions. As the embryo continues to develop, these three regions remain distinct. From the continuity and the heaviness of

the wall which marks off the basal region, it is to be concluded that this is the first transverse division wall. The other, judging from similar evidence, is apparently the result of a transverse division in the epibasal cell. For indication of such divisions note figs. 5, 6, 7, 9, 10, 13. It will be observed that the division which marks off the basal region is the more prominent, probably representing the plane of the first transverse division.

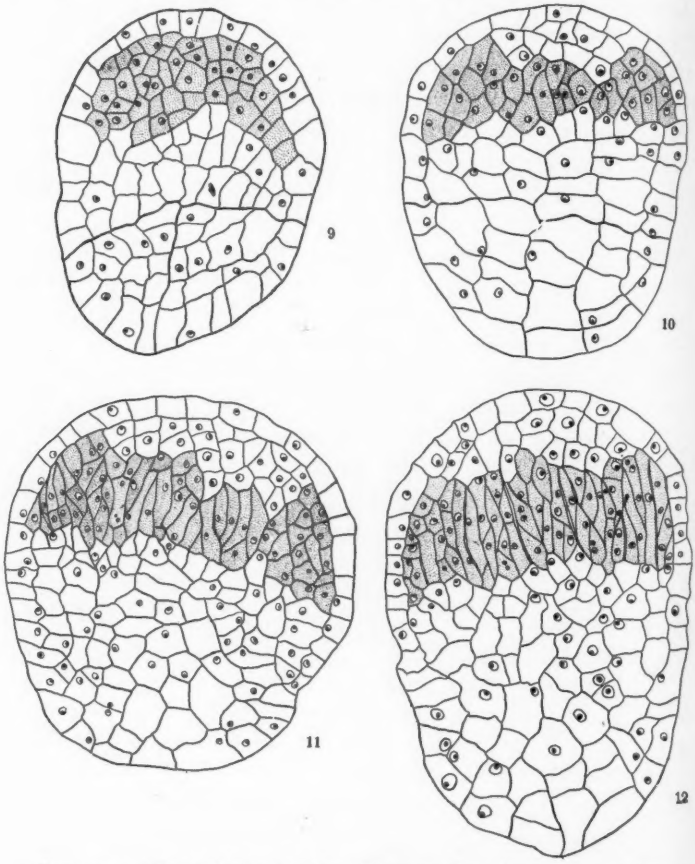
Within the three regions of the embryo anticlinal divisions occur, then periclinal divisions; and then anticlinal, periclinal, and radial divisions in irregular sequence. In a large number of embryos a triangular cell was observed at the apex. That it has a definite function as an apical cell, as is suggested by DUPLER (9) for *Reboulia haemispherica*, is not certain. If so, it seems clear that it does not so function for any considerable length of time.

As divisions continue, and while the embryo is still subspherical in form, a change occurs in the staining reaction of the cells, those in the distal region taking a somewhat deeper stain (fig. 6). With continued division, the cells in the apical region take the heaviest stain, and in the other two regions the staining reaction varies, being a little heavier in the middle region than in the basal (figs. 7, 8). The density becomes greatest in the central cells of the apical region, but the cells in the peripheral layer of this region do not increase in density so rapidly. These outer cells are the ones which will form the capsule wall (fig. 8).

As the density of the inner cells of the apical region increases, they begin to elongate in a direction parallel to the axis of the sporophyte (fig. 11). At the apex there is a small group of cells which remain approximately isodiametric (figs. 10, 11), and take about the same amount of stain as the cells of the capsule wall. This cap of sterile cells persists throughout the life of the sporophyte and undergoes no noticeable change until the walls of these cells take on thickenings similar to those of the capsule wall.

With the continued elongation of the dark-staining (potentially sporogenous) cells, there are cells at the base of this region which do not elongate, and which take a stain heavier than that of the cells in the region of the stalk, but approximately resembling in this respect the cells of the capsule wall. In consideration of the later appear-

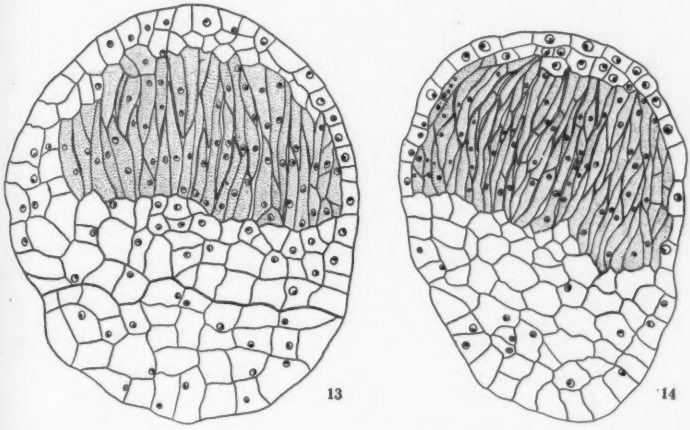
ance of capsule wall thickenings in this region of the mature sporophyte, it is probable that the cells in question are those which



FIGS. 9–12.—Fig. 9, further division, different regions becoming more distinct; first transverse division, marking off foot, conspicuous; fig. 10, first appearance of sterile, lighter staining cells at apex (to become sterile cap); fig. 11, early elongation of cells of sporogenous region; fig. 12, further elongation of binucleate cells of sporogenous region; sporophyte longer than broad.

are to form the part of the capsule wall extending across the base of the capsule.

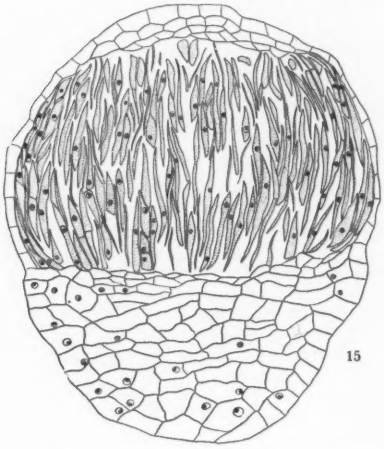
The sporogenous cells elongate greatly (figs. 12, 13). The sporogenous region broadens somewhat and many of the much elongated sporogenous cells are observed to be binucleate, and in some instances appear to contain more than two nuclei (figs. 12, 13). These cells begin to separate from one another laterally (fig. 14). At first no difference between cells that are to become elaters and the true sporogenous cells is to be observed. When the cells are well separated from one another (fig. 15) there appears a difference in

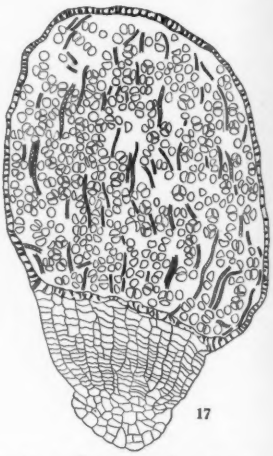


FIGS. 13, 14.—Fig. 13, cells of sporogenous region elongated still more, with tendency to separate from one another; conspicuous transverse division marking off foot; fig. 14, further elongation; cells of sporogenous region dividing by oblique transverse wall, to result in uninucleate cells.

width, some being broader than others. All are uninucleate at this time. Those cells which are more broad continue to increase in size, the others remaining very long and narrow. The narrow cells are scattered between the broader ones, being arranged in no definite manner. In the broader cells, which are the true sporogenous cells, transverse cell divisions occur, so that in each a row of from four to eight spore mother cells is formed. These spore mother cells are irregular in shape and are flattened where they come in contact with one another. At this time the capsule is increasing in size, cell division and elongation having occurred in the cells of the capsule

wall. The spore mother cells increase slightly in size, and a very heavy wall is formed around each row and between the spore mother





FIGS. 15, 17.—Fig. 15, cells of sporogenous region well separated; narrow and broader cells observable; note row of small somewhat flattened cells at base of region (to become wall across base of capsule); fig. 17, sporophyte approaching maturity; foot globular; many transverse divisions in seta; thickenings on capsule wall; mature elaters with spiral thickenings; spores separating from tetrads.

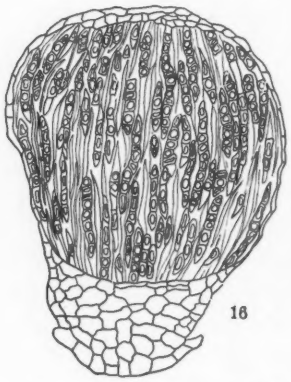
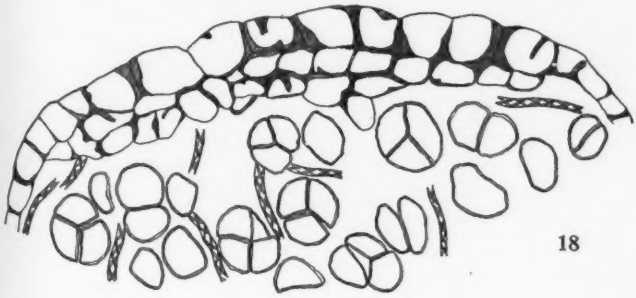


FIG. 16.—Great increase in size of capsular region; spore mother cells with heavy walls in rows; narrow elaters between.

cells within the rows (figs. 16, 21). The elaters remain very thin and inconspicuous as regions of slightly granular cytoplasm, with extremely thin walls, lying between the heavy-walled, conspicuous rows of spore mother cells. The spore mother cells now increase in size, and at about the same time the heavy walls which surround and separate them begin to disappear. This disappearance of the walls seems to be a dissolving process, proceeding from the outside inward, until there remain only faint strands of the old wall substance between the spore

mother cells. In the meantime a new cell wall has been forming around each spore mother cell (fig. 22). This new wall, although it never becomes thick like the old wall which has dissolved, is very



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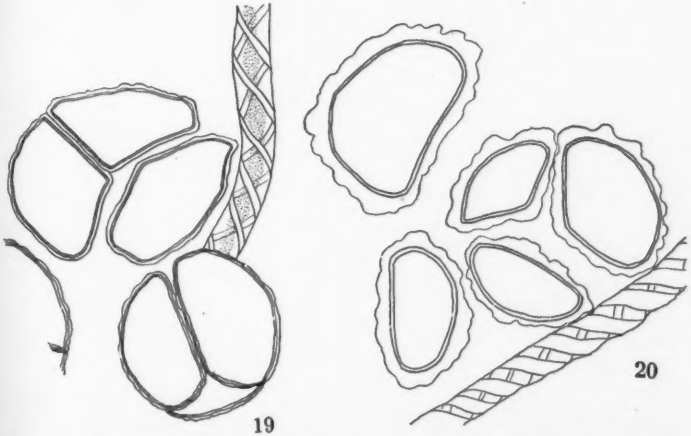


19 20

FIGS. 18–20.—Fig. 18, apex of almost mature sporophyte, showing thickenings on walls of cells of sterile cap; fig. 19, spores still in tetrads, with walls newly forming; cytoplasm still present in elaters in form of narrow strand down center; thickenings of elaters rather narrow; fig. 20, spores mature with thickened walls; elaters mature with broad spiral band thickenings and no cytoplasmic content.

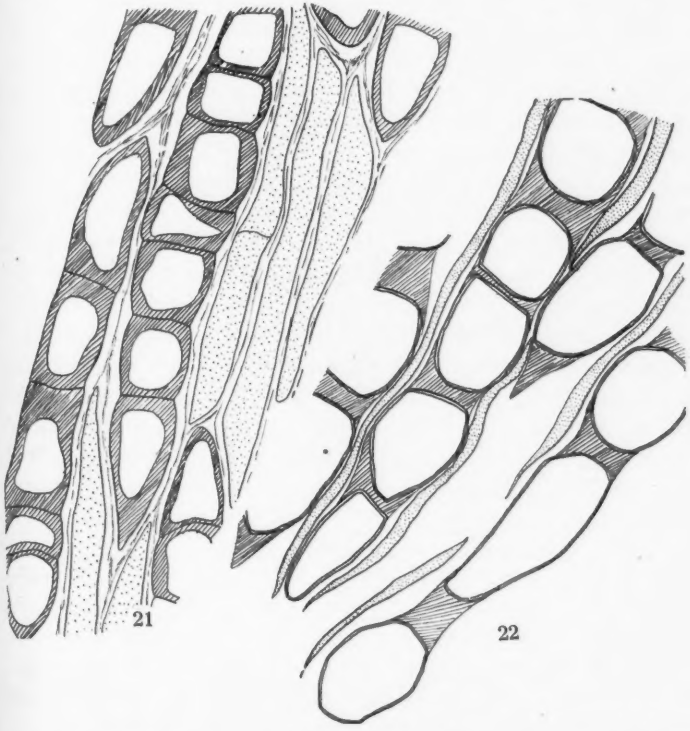
definite. The spore mother cells remain in the original rows until after nuclear divisions have occurred within them, being held there apparently by traces of the old dissolved walls. Each spore mother cell, having increased somewhat in size, now divides to form a tetrad of spores. These spores develop the characteristic

walls consisting of three layers, endospore, exospore, and irregular epispore. The spores remain together in tetrad formation until their walls are well formed (figs. 18, 19).

Not until nuclear division has occurred within the spore mother cells do the elaters begin to develop their characteristic thickenings. Up to this time the cell wall of each elater has been thin and its contents have been faintly granular. As the thickenings (spiral bands) begin to appear, the cytoplasm seems to lie in a strand running longitudinally through the center of the cell (fig. 19). The cytoplasm gradually disappears, and at the same time the thickenings of the wall become wider and more definite. The end result, at the time when the spores separate from the tetrad, is a very long, apparently empty cell with two spiral band thickenings on the inner side of the wall (fig. 20). These spiral bands run in opposite directions, so that they cross each other at regular intervals, giving a braided effect when looked at superficially.

At about the time of completion of the nuclear divisions in the spore mother cells, the first indications of thickening on the walls of the cells of the capsule wall begin to appear. These thickenings continue to grow until they form narrow girdling bars, which almost completely encircle the elongated cells of the capsule wall. The number of these thickenings in a given cell varies, four or five being the average. At the apex of the capsule they are very wide and heavy. Not only do they occur in the cells of the capsule wall, but also in those of the apical group of sterile cells within the capsule wall (fig. 18). This small group of cells, consisting of two or three layers of cells, has persisted since the time of their first differentiation (fig. 11). The thickenings in this region are not as heavy, nor do they encircle the cell in as regular fashion, as those in the cells of the capsule wall. Often they are in the form of short bars, tapering at each end. Thickenings of the walls also occur in the layer of cells at the base of the capsule wall. This irregularity in appearance is probably due partly to the fact that the cells of this layer are not as consistent and regular in shape as those of the outer capsule wall. It is also due to the form of the thickenings themselves, for, when compared with the thickenings of the capsule wall cells, they are narrower, not so long, and occur less frequently in a given cell.

While the sporogenous cells are dividing and elongating, the cells of the foot and seta are also dividing. The cells of the lowest layer of the foot are large, have large nuclei, and at a later period take a dense stain, in accordance with the usual condition of such haus-



FIGS. 21, 22.—Fig. 21, spore mother cells in rows with very heavy walls; fig. 22, new walls being formed around spore mother cells; old walls gradually disappearing; narrow elaters occurring between rows of spore mother cells.

torial cells. In the majority of cases the foot as a whole is rather globular in shape, and consists of a few cells.

At the time of the first elongation of the sporogenous cells, divisions occur in the region of the seta so that the sporophyte from this time on is longer than broad (fig. 12). Divisions within this seta

region are relatively few for some time, being just sufficient to keep pace with the general increase in size of the whole sporophyte. But at the time when the spores are separating from one another in the tetrads, many transverse divisions occur in the cells of the region of the seta (fig. 17). As a result of the elongation of these newly formed cells the capsule, at complete maturity, will be pushed out through the surrounding calyptra and perianth.

During the development of the sporophyte, the cells of the venter of the archegone have divided, keeping pace with the growing embryo. This division of cells is begun immediately after fertilization has occurred and continues until the spores are formed, thus forming the calyptra. At the same time divisions have occurred in the tissue below the base of the archegone, resulting in the formation of a broad massive cushion, which pushes the developing sporophyte farther out from the archegonial head. As this division begins, a collar of cells at the base of the archegone begins to divide, forming a single-layered sheath of cells, the perianth. Just as in *Marchantia polymorpha*, the developing sporophyte is then surrounded by the old wall of the archegone, the calyptra, a sheath, the perianth, and the involucre, which incloses the whole group of archegonia and developing sporophytes beneath each lobe.

Discussion

Following the first transverse division of the embryo, the formation of vertical walls to form quadrants is not observed in *Marchantia chenopoda*. In a few cases in later embryos there are some indications that the original divisions formed quadrants of this nature; but on the other hand there are many embryos in which there is no possible suggestion of a quadrant or octant division.

The division planes which are conspicuous are those of two transverse divisions, one being the first division of the zygote, the other a division of either the hypobasal or the epibasal cell. These divisions remain conspicuous for some time. The lower one, which may be observed at a very late period, appears to separate the foot from the rest of the sporophyte. If one may assume that a wall which is continuous and is somewhat heavier than other walls is the first division wall, then this wall which marks off the foot from the region

of the seta and capsule is the first division wall. Whether the capsule and seta are separated from each other by the second transverse division is not to be so easily demonstrated in the later stages. In the earlier stages, however, there is considerable evidence that this is the case.

To return to the three regions, basal, middle, and apical, as divisions occur in these regions and there appears a difference in the staining reaction (fig. 7), it is to be observed that the cells of the basal region are most lightly stained, those of the middle come next in density, and those of the apical region are the most dense. The apical and middle regions are to be distinguished not only by the continuous cell wall, separating them, but also by a difference in cytoplasmic content. As divisions continue and density increases, the central cells of the apical region, destined to become the sporogenous tissue, are the most dense; those at the periphery of the apical region, which are to give rise to the capsule wall, are a little less dense; and the middle and basal regions show about the same ratio in staining capacity to one another as previously, both regions being lighter than the apical region.

As the embryo increases in size and the sporogenous cells become more dense in cytoplasmic content, the division wall which marks off the foot remains conspicuous. Between this line and the sporogenous region there is a region which corresponds favorably with the earlier middle region, and also with the seta of later stages (figs. 9, 10). As these regions are followed in the succeeding development, it is evident that the sporogenous region, with its outer row of cells destined to become the capsule wall, develops into the capsule; that the lower part, the earlier basal region so clearly marked off by the early division wall, becomes the mature foot; and that the part between, the earlier middle region, marked off definitely from the foot and somewhat vaguely from the capsular region, is to become the seta.

The history thus outlined does not agree with that reported for *Marchantia polymorpha* (10), nor with that in many of the other Marchantiales, in which the first divisions result in quadrants. This quadrant type of embryo has been reported for *M. polymorpha* by Durand (10). It is also reported for *Riccia*, *Targionia*, and *Fim-*

briaria californica by CAMPBELL (3), for *Corsinia* by MEYER (18), for *Cyathodium foetidissimus* by LANG (16), for *Cryptomitrium* by ABRAMS (1), and for *Conocephalum* by CAVERS (4).

The embryo of *Reboulia haemisphaerica* suggests in some ways that of *Marchantia chenopoda*, in that it is at first a filament of three or four cells (9, 13, 22). Such an embryo is also described for *Plagiochasma* by STARR (20), for a species of *Targionia* by O'KEEFE (19), and for *Geothallus* and *Sphaerocarpos* by CAMPBELL (3). In *Conocephalum*, according to CAVERS (4), a second transverse wall sometimes appears in the epibasal cell, although the usual condition is for vertical walls to divide the embryo into octants. GARBER (12) found a similar condition in *Ricciocarpus*, in which the usual occurrence was that of the octant form, but occasionally a row of three cells was observed.

In *Marchantia chenopoda*, it appears that after the first division, which forms the hypobasal and epibasal cells, another transverse division occurs in one of these. Whether this division is in the hypobasal cell has not yet been demonstrated conclusively, since no mitotic figures have been observed. It has been reported by DURAND (10) for *M. polymorpha* and by KIENITZ-GERLOFF (14) for *Preissia* that the hypobasal cell develops into the foot and seta, and the epibasal cell develops into the capsule. In *M. polymorpha* this decision is based apparently on the presence of a conspicuous transverse division between the regions of the seta and the capsule, and upon the fact that the staining reaction is such in the early stages that it appears that one half becomes capsule and the other half becomes foot and seta. On this same basis, it is to be noted in *M. chenopoda* that the conspicuous division wall (first?) is the one marking off the foot from the seta; and that at an early age the sporogenous, heavier staining region occupies not one-half, but approximately one-third of the young sporophyte.

This last point would tend to suggest that there might be a difference in the early embryogeny of the sporophytes of these two forms, for at the time when the sporophyte of *M. polymorpha* is still distinctly spherical in form, the sporophyte of *M. chenopoda* has elongated parallel to the long axis of the archegone. This elongation is apparently due to division and growth in the middle or seta region.

The larger number of cells in the region of the seta, as compared with *M. polymorpha*, is a difference which persists until the time when the true sporogenous cells and elaters are first to be differentiated in the capsular region. As a result, during this period of time, the sporophyte of *M. polymorpha* is broader than it is long, while that of *M. chenopoda* is longer than it is broad.

It is very important that it be noted that the sequence of divisions and development just outlined is not the one and only occurrence. Frequently the first divisions are extremely oblique. In such instances the differentiation of tissues occurs in the same general region (in relation to the position in the archegonium), but it does not follow the first division walls. DURAND makes clear that this also occurs in *M. polymorpha*. But when the walls are not too extremely oblique the succeeding divisions and development follow the description already given.

The occurrence of sterile cells at the apex of the capsule is a condition not reported by DURAND for *Marchantia polymorpha*. MEYER (18) reports such a sterile region, however, and CRIBBS (6), who also found a columella in one case, reports a sterile cap of cells in *M. polymorpha*. There is no indication of a columella in *M. chenopoda*. The sterile cap, consisting of two or three layers of cells, appears at a time when the cells of the sporogenous region are first beginning to elongate. It remains intact, increasing slightly in size, with the increase in size of the whole sporophyte. In the mature sporophyte the cell walls of this region become thickened in a manner similar to that characterizing the cells of the capsule wall.

This condition suggests that of *Conocephalum*, *Lunularia*, and *Dumortiera*, in which, according to CAVERS (5), there is a "well-developed apical thickening which is thrown off as a lid." In these forms the lower portion of the capsule splits into four to eight teeth, after the lid has fallen off. The dehiscence of the capsule of *Marchantia chenopoda* has not been observed. Such a thickened apical cap is also reported by HAUPT (13) for *Reboulia haemisphaerica* and *Preissia quadrata*. For *Marchantia*, CAVERS (5) reports that the apical cap is only indicated by "an imperfect or loose layer of cells lying within the normally single-layered capsule wall at the apex, and the capsule opens by teeth extending to the apex."

The foot presents another variation from *Marchantia polymorpha*. In *M. chenopoda*, in the majority of cases, it is bulbous in shape and consists of a few cells. In *M. polymorpha* the foot is "anchor-shaped," and usually made up of more cells. Occasionally, however, there appears in *M. chenopoda* a foot which shows a slight tendency to be somewhat anchor-shaped, but this is never as marked as in *M. polymorpha*. It seems that this occasional appearance of a differently shaped foot must indicate a condition that is not fixed; that, rather, there is here expressed a tendency to reduction in the foot, the haustorial activity being carried on by a few large cells, rather than by many smaller ones. The similar bulbous foot found in *Reboulia* by HAUPT is considered by him as one of the primitive features of the genus. There is the possibility that this is the condition in *M. chenopoda*. It seems strange, however, that in both *Reboulia* and *M. chenopoda* the advanced condition of a thickened sterile cap at the apex should be combined with a primitive condition in the foot. Because of this fact there is a tendency to conclude that the smaller foot of *M. chenopoda* indicates a reduction, rather than a primitive condition.

When the cells of the sporogenous region are well elongated, many of them are observed to be binucleate and sometimes multinucleate. This condition persists for some time, but when the cells become well separated from each other, and the elaters and true sporogenous cells can be distinguished because of the difference in size, all the cells appear to be uninucleate. It is probable that the binucleate condition is one which exists only until the ultimate division forming the elaters and the sporogenous cells, each of which will form a single row of spore mother cells.

Summary

1. The first transverse division of the zygote in *Marchantia chenopoda* separates the foot from the seta and capsule; that is, the foot develops from the hypobasal cell, the seta and capsule from the epibasal cell.

2. Two primary transverse divisions seem to be the regular occurrence in the embryo, instead of a quadrant division. These divide the embryo into apical, middle, and basal regions. The basal region

develops into the foot, the middle into the seta, and the apical into the capsule.

3. The sporophyte, soon after the first difference in staining is observed, becomes longer than it is broad. This is due to divisions in the region of the seta. In *M. polymorpha* the cells of the stalk remain relatively few in number, while the rest of the sporophyte goes on dividing, the result being that during the same period of development as in *M. chenopoda* the sporophyte is broader than it is long.

4. The sterile cap of cells present at an early stage in the capsule persists throughout the life of the sporophyte; the walls of these cells, when mature, taking on thickenings similar to those of the cells of the capsule wall.

5. The cells which are to form the layer of cells across the base of the capsule are distinguished by their staining reactions at the time of the first differentiation of the capsule wall cells from the sporogenous cells.

6. Many of the cells of the sporogenous region, during early elongation, are binucleate.

7. The foot is bulbous in shape and occasionally shows a slight tendency to be "anchor-shaped." Such a condition probably indicates a reduction in this region.

8. The antherid and archegone show the same fundamental structure and development as in *M. polymorpha*.

The writer wishes to express grateful appreciation to Professor GEORGE S. BRYAN for his advice and encouragement at the beginning of the study, and to Professor C. E. ALLEN for his suggestions and careful criticism.

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[*Accepted for publication January 12, 1929*]

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MORPHOLOGICAL STUDIES ON A NEW SPECIES OF MARCHANTIA

ENID A. HEBERLEIN

(WITH TWENTY-ONE FIGURES)

In this study the investigation has been concerned mainly with the development and structure of the sporophyte of a species of *Marchantia* which is as yet undescribed. The development of the sex organs has also been traced. The history of the development of the sporophyte, and to some extent that of the sex organs, is followed and compared with the corresponding history for *M. polymorpha*.

Materials and methods

Material of this species was collected by Professor GEORGE S. BRYAN in several localities in the province of Huanuco in Peru. The particular material used in the present study was found at Cani, a village about 18 miles east of the town of Huanuco, the capital of the province.

The plant looks much like *Marchantia polymorpha*, with its flat thallus and dichotomous branching. The successive forks in the branching are usually 1.5–2.5 cm. apart. The margin is entire or slightly undulate. Ventral scales are in two distinct rows on the median riblike portions. Their shape is spatulate. The male receptacle is borne on a stalk 1–1.5 cm. high, and when mature is 3 cm. broad. There are five to eight rays in palmate arrangement, the basal sinus being almost a straight line.

The female receptacle is borne on a stalk 3 cm. long, and at maturity is 5–7 mm. broad. There are four to eight rays which are so poorly defined as to be almost indistinguishable. The general outline when mature is that of a skull cap. Gemmae cups are numerous.

The material was killed in the field at various times during the months of April, May, and June, 1923. The killing fluid used was prepared according to the following formula: 1 cc. of glacial acetic acid, 1 gm. of chromic acid, and 400 cc. of water.

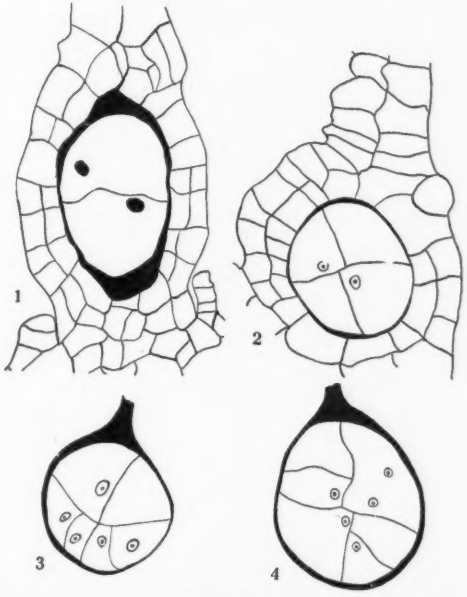
After eight months the material was removed from the fixing fluid, washed, and run through a close series of alcohols to 70 per cent. At this stage it was turned over to the writer. It was fully dehydrated and then cleared in an alcohol-chloroform series to pure chloroform. Then it was imbedded in paraffin, sectioned at thicknesses of 5 to 10 μ, and the sections stained with safranin in combination with light green.

Development of sporophyte

The first division in the zygote is usually transverse (figs. 1, 3), or in some cases is somewhat oblique (figs. 2, 4). This first division wall is apparent later, and may be traced for several stages in the sporophyte. The plane of the second division is almost perpendicular to that of the first, dividing the embryo into quadrants (fig. 2). At this time the embryo broadens out, becoming spherical or somewhat ovoid. The next divisions vary in different embryos. In fig. 3 the two lower quadrants are divided by anticlinal walls, while in fig. 4 the divisions following the second are in diagonally opposite quadrants. If the embryo has broadened out considerably, the corresponding division is in a plane parallel to that of the first vertical division (fig. 5). A few more anticlinal divisions occur, and then periclinal walls begin to be formed (fig. 6). Further divisions are periclinal and anticlinal without definite sequence, until a spherical mass of cells is produced in which the primary division walls can be recognized clearly (figs. 7, 8). The embryo has increased but little in size up to this time, so that its component cells become successively smaller as division progresses.

While this development is proceeding in the embryo, changes are taking place in the venter of the archegonium and in the tissue at its base. By transverse divisions this basal tissue grows out rapidly until it forms a tubular sheath, the pseudo-perianth, surrounding the venter. This is a single layer of cells in thickness. The beginning of this sheath is shown in fig. 1. Another change is noted in the wall of the venter and in the base of the neck. Periclinal divisions occur until two or three layers of cells are formed around the young embryo; the calyptra thus formed serves as a protective covering for the growing embryo (figs. 1, 5).

The embryo and surrounding tissues now enlarge rapidly, the embryo being for a time nearly spherical (fig. 9), and later broader than long (fig. 10). The first indication of a differentiation within the embryo sporophyte is a change in the staining capacity of the

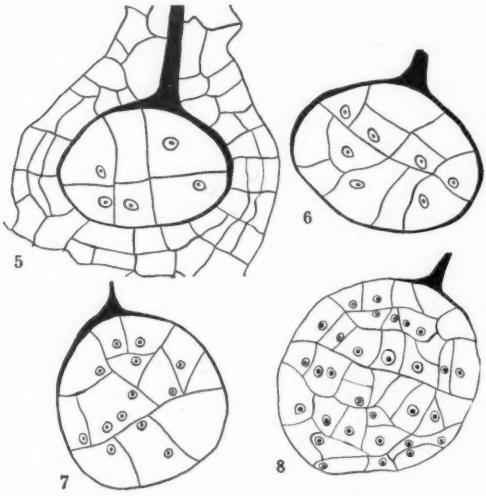


FIGS. 1–4.[1]—Fig. 1, transverse division of zygote and beginning of pseudo-perianth; fig. 2, embryo divided into quadrants, periclinal divisions in venter wall; fig. 3, one type of further development of embryo after quadrant; fig. 4, another type of development following quadrant stage.

cells. Those in the distal half, which are to form the capsule, become richer in protoplasmic content, while those of the proximal half (the future seta and foot) take less stain (fig. 9). The two portions thus differentiated are usually separated by the first transverse division wall. This is very plain in fig. 9. In fig. 11 there is in part more than

[1] All figures were drawn with the aid of a camera lucida at table level, and show the following magnifications: figs. 1–11 ×700; fig. 12 ×555; fig. 13 ×325; fig. 14 ×250; figs. 15, 17, 20 ×1150; fig. 16 ×100; fig. 18 ×120; fig. 19 ×140; figs. 21, 22 ×690.

one layer of cells below the potential sporogenous tissue which does not take deep stain, but is very similar to the single peripheral layer of cells in the upper part of the capsule, which cells will become the outer sterile wall of the capsule. It is likely that these cells not only form the inner boundary of the capsule, but may also contribute to some extent in the formation of the stalk.

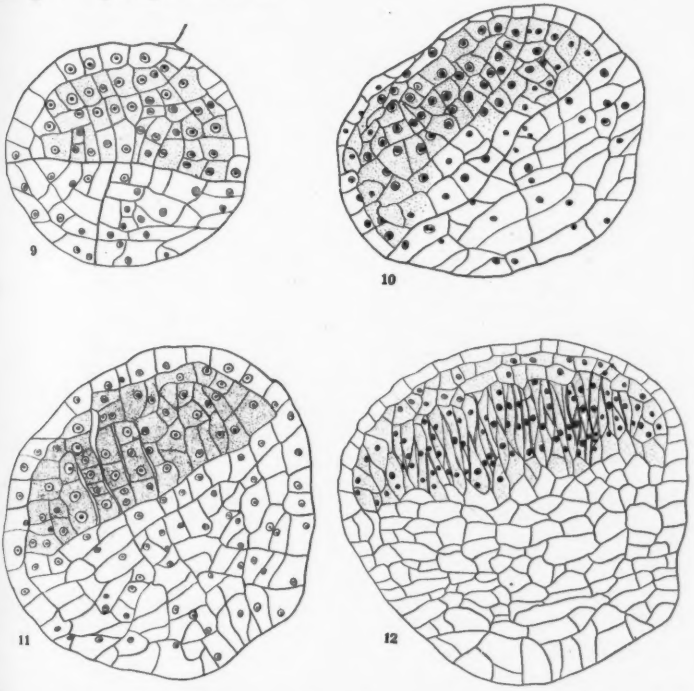


FIGS. 5–8.—Fig. 5, third type of development following quadrant, further divisions in venter wall; fig. 6, first periclinal walls appearing; figs. 7, 8, further periclinal and anticlinal walls.

The cells of the sporogenous tissue are at first irregularly isodiametric and similar to the cells of the seta (fig. 10). Then they elongate somewhat in a direction parallel to the axis of the archegonium, and there is a tendency in the rows of elongated cells to separate slightly from one another (fig. 11). A slight bulging of the basal cells of the embryo indicates the beginning of the foot.

In fig. 12 a more advanced condition is shown. The foot has become a little more prominent, but has not yet assumed its final shape. The capsule portion is broad and the potential sporogenous cells are numerous, plainly elongated and often binucleate. At this stage also a cap of cells, destined to remain sterile, can be observed

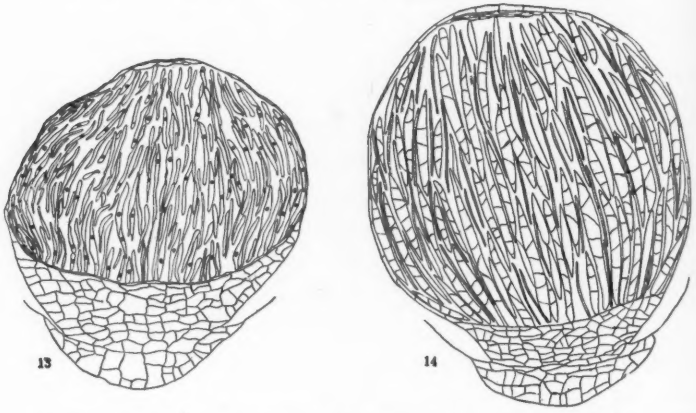
in the apical region just beneath the capsule wall. The cells of this cap are approximately isodiametric, thus resembling the sporogenous cells at an earlier stage, and are less deeply stained than the elongated sporogenous cells.



FIGS. 9–12.—Fig. 9, first differentiation of sporogenous tissue more deeply stained; fig. 10, sporophyte broader than long; fig. 11, beginning of separation longitudinally of sporogenous cells; fig. 12, further separation (note binucleate cells and sterile cap).

In a still older sporophyte (fig. 13) the foot has penetrated more deeply into the tissue at the base of the archegonium, and expanded laterally, forming an anchor-shaped absorbing organ whose cells are filled with deeply staining material. The cells of the sporogenous tissue have separated completely from one another and have elongated still further. The elongated cells are now uninucleate; evidently transverse cell divisions have occurred since the stage of fig. 12.

A few of these elongated cells are narrower than the others, and this is the first indication of a differentiation between sporogenous cells and elaters. At this stage, however, the difference is barely noticeable. At a somewhat later stage (fig. 14) the distinction between sporogenous cells and elaters is much more marked, the sporogenous cells having become larger and the elaters remaining comparatively very slender. The two kinds of cells alternate irregularly. The cap of sterile cells is still visible in the stages shown in both fig. 13 and fig. 14. The cells composing it have become much

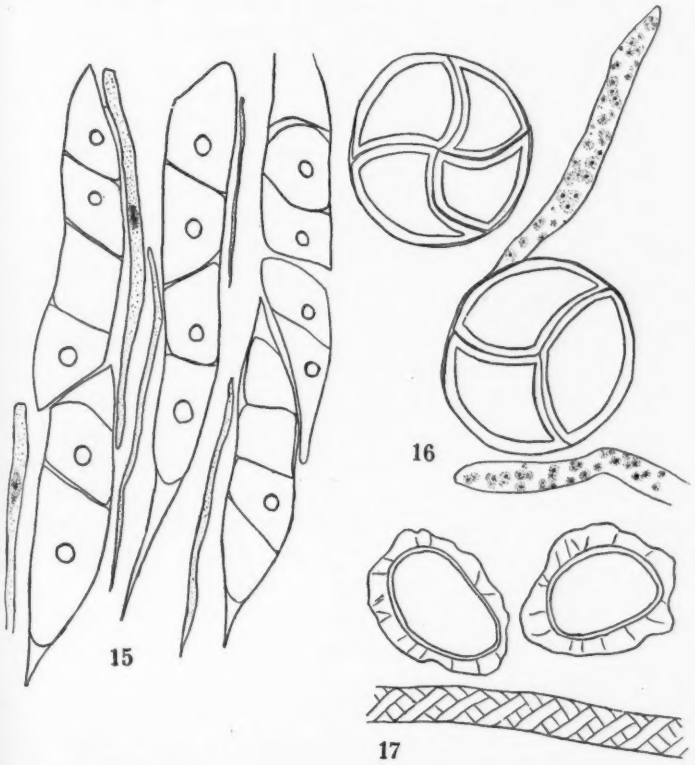


FIGS. 13, 14.—Fig. 13, sterile cap still present, elaters more slender than sporogenous cells; fig. 14, rows of spore mother cells alternating irregularly with elaters, sterile cap.

flattened against the capsule wall and seem shrunken. At this time the seta is very short and its cells are broad. The capsule has become much larger in proportion to the stalk and foot regions, and is almost spherical in outline.

The sporogenous cells, after increasing in size, become divided by transverse walls to form rows of two to eight cells each, all still inclosed by the old wall of the elongated cell (fig. 15). The cells composing these rows are the spore mother cells. They are irregular in shape, being flattened against the old walls of the mother cells and also where they are contiguous with one another. Each of these spore mother cells becomes rounded and later divides to form four spores.

These spores remain in tetrads for a time (fig. 16). The elaters at this time are still granular in content, but the granules have become aggregated into groups. By the time the spores are mature and have

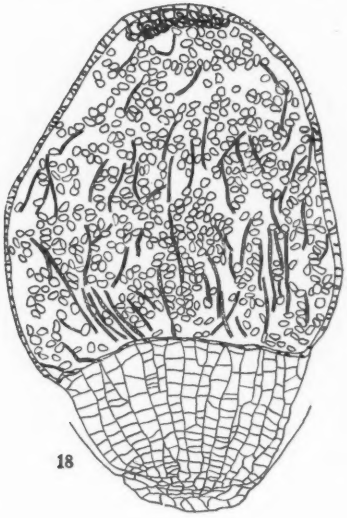


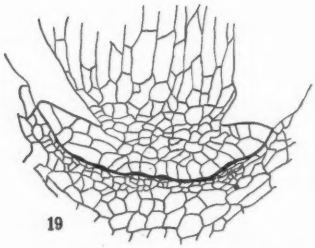
FIGS. 15–17.—Fig. 15, detailed drawing showing rows of spore mother cells and slender elongated cells, young elaters; fig. 16, tetrads of spores derived from spore mother cells and elaters with walls still unthickened; fig. 17, mature spores and elater.

separated from their tetrad grouping the elaters have matured, having formed spiral bands on the inside of the wall (fig. 17).

The nature of the material prevented full working out of the detail of the development of elaters. They seem to have developed from elongated cells among the sporogenous cells as already stated.

The contents are at first uniformly granular; then the granules aggregate, and later become empty cells in which some material, possibly the granules, has been deposited in two spiral bands which are regular and uniform, running the length of the long elater.





FIGS. 18, 19.—Fig. 18, details of mature sporophyte; fig. 19, detail of foot of mature sporophyte.

Fig. 18 shows a mature sporophyte. At this time the foot is small in proportion, still keeping its anchor shape; however, the cells of the seta have grown and divided, so that the capsule has been pushed up through the calyptra, and there is a marked thickening on the walls of the sterile cells of the capsule wall and of the cap of the capsule. The only other change in the sporophyte after this stage is an elongation of the much divided cells of the seta (fig. 19), so that the capsule is pushed out still farther.

Sporophyte

The position of the first division wall in the zygote varies within narrow limits. It may be transverse; in fact, in all the preparations in which the first division only had occurred the plane was horizontal. But to judge from some later stages of the embryo, this first division may be obliquely transverse (figs. 2, 4, 6). This first division wall may be followed through the development of the embryo to a stage as late as that of fig. 11, and, in general, this wall seems to divide the sporophyte into a distal portion which will become the capsule, and a proximal portion which gives

rise to the seta and foot. This is usually the case in *Marchantia polymorpha* also (4).

Succeeding divisions form quadrants, and then anticlinal and periclinal walls form a mass of cells which is almost spherical. As in *M. polymorpha* (4), there is an increase in size; then a stage in which the embryo is broader than long (fig. 10); and still later it becomes longer than broad (figs. 13, 14). The last mentioned growth in length does not occur until the sporogenous cells have elongated considerably; thus it is the capsular region which at first increases in length. Later the seta elongates, and the sporophyte becomes much longer than broad.

The sporogenous tissue is first differentiated by a change in staining capacity. The isodiametric cells of this distal region take a heavy stain. A single peripheral layer of cells in the capsule portion takes a lighter stain. The other cells elongate, often become binucleate (figs. 11, 12), and later by divisions become uninucleate again. These elongated, heavily staining cells are the ones from which elaters and the spore mother cells will arise. There seems to be further elongation, and then the elaters remain slender while the other, now broader, cells divide transversely to form each from two to eight spore mother cells. DURAND (4) has not followed this part of the history in much detail, but says that the long sporogenous cells may be divided by transverse or by transverse and longitudinal walls into groups of eight, rarely four, cells. Thus there may be formed in *M. polymorpha* biseriate or uniseriate rows of spore mother cells. It may be noted that only transverse divisions are observed in the species under study here. As in *M. polymorpha*, the spore mother cells divide, each to form four spores which remain in the tetrad arrangement for a time. During this time the elaters have been developing from slender elongated cells to mature form with spirally thickened walls, but the process of tracing this development met with difficulty and the exact history in detail is lacking.

There is a sterile cap of cells just beneath the capsule wall in the apical region. This cap of two or three layers of cells appears at a time when the cells of the sporogenous region first elongate, is present but greatly compressed at the time the spore mother cells are formed but still arranged in rows (fig. 14), and is still to be seen

in the mature sporophyte, where the walls of the cells composing it are thickened spirally and with bands as are the cells composing the sterile wall of the capsule (fig. 18). DURAND does not report such a cap. CRIBBS (2) did find a sterile region of cells in *M. polymorpha*. CAVERS (1) reports that in *M. polymorpha* "the apical cap is only indicated by an imperfect or loose layer of cells lying within the normally single-layered capsule wall at the apex."

In *M. polymorpha*, as described by DURAND, the foot becomes anchor-shaped. The cells of the seta are in quite regular rows; they are at first broader than long and later become much elongated (fig. 19). Since the capsule is fully mature and the stalk is still short (fig. 18), it is probable that the elongation of the stalk is coincident with the dehiscence of the capsule. Whenever a long stalk is found the capsule has discharged its spores and is losing its shape.

Sex organs

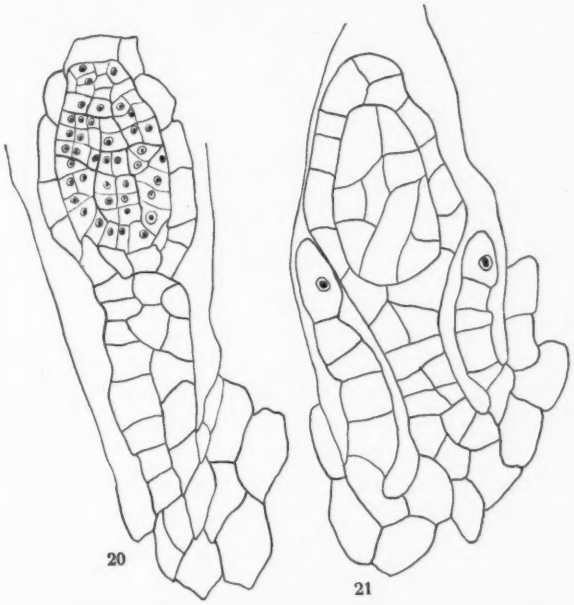
During this study it has been found that in this as yet unnamed species of *Marchantia* the development of the antheridium follows in general the corresponding history in *M. polymorpha*. The points of difference noted were the length and size of the stalk and the presence of what may be called paraphyses (6). In the species under investigation the stalk has four tiers of large cells while the stalk of *M. polymorpha* consists of two small cells in approximately similar stages in development. The divisions in the stalk cells occur later in *M. polymorpha*, and ultimately there is formed a stalk of five or six tiers of cells. The stalk in this unnamed species finally becomes six or seven cells long and quite massive, and it is noticeable that the character of relatively larger size is early manifest (figs. 20, 21).

The other noticeable feature was the outgrowth at the base of the young antheridium which elongated and in some cases became two- or three-celled. These paraphyses grow up around the organ in its cavity (figs. 20, 21). In later stages only traces of such cells are found and their function is unknown (6).

The development of the archegonium is very regular, and compares almost exactly with that of *M. polymorpha*. There are usually four neck canal cells, but five are not so uncommon in this species as in *M. polymorpha*.

Androgynous receptacles

During the sectioning of this material two androgynous receptacles were discovered. These had much the shape of the female receptacles. The archegonia were borne on the under side as they ordinarily are, and the antheridia were in cavities opening to the upper side. Cutting (3) found some androgynous receptacles on a



Figs. 20, 21.—Fig. 20, maturing antheridium with long stalk and long mucilaginous hairs; fig. 21, more mature stage of antheridium with massive stalk evident.

hybrid species of *Marchantia;* but in that instance the antheridia were borne on proliferations from the under side of the receptacle, while the archegonia were in the ordinary position. The occurrence of androgynous receptacles in this new species may indicate a tendency toward a monoecious condition. There was nothing abnormal in the appearance of the receptacles or in the number or arrangement of the sex organs.

Summary

1. This study deals with an unnamed species of *Marchantia* growing in the Peruvian Andes. Male and female receptacles were studied and the development of the sporophyte and sex organs was traced.

2. The first division in the zygote is transverse or obliquely transverse, dividing the embryo into a distal portion which will become the capsule and a proximal region which will become foot and seta.

3. The second division is perpendicular to the first.

4. Periclinal and anticlinal divisions form a spherical mass of cells. The distal region takes a deeper strain and later forms sporogenous cells.

5. A peripheral layer of cells around the sporogenous cells forms the capsule wall; the walls of these cells later show bandlike thickenings.

6. A cap of sterile cells is present in the apical region from the time the cells of the sporogenous region first elongate until the sporophyte is mature. These cells also have thickenings.

7. At one stage the elongated sporogenous cells are binucleate, but by divisions soon result in uninucleate cells, and these give rise to rows of spore mother cells.

8. The stalk remains short until the spores are mature, and then the cells elongate and push the capsule out through the calyptra.

9. The antheridium has a typical development and also has paraphyses and a noticeably long and massive stalk.

10. The archegonium development is typical of *M. polymorpha*.

11. Two androgynous receptacles with both kinds of sex organs in their ordinary positions were found.

The writer wishes to express grateful appreciation to Professors GEORGE S. BRYAN and C. E. ALLEN for suggestions and criticisms in the progress of this study.

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[*Accepted for publication January 12, 1929*]

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COMPARATIVE EFFECT OF TEMPERATURE ON RATE OF PURE CHEMICAL REACTIONS AND RATE OF SUGAR UTILIZATION BY A PLANT AND A COLD BLOODED ANIMAL

G. C. WICKWIRE, L. D. SEAGER, AND W. E. BURGE

(WITH TWO FIGURES)

It is recognized that temperature influences the rate of chemical reactions, a rise in temperature increasing the rate and a fall in temperature decreasing it. A number of investigators have studied the effect of temperature on the rate of the chemical reactions of several different chemicals, and found an increase in rate varying anywhere from 1.2 times to as high as 3.6 times for a rise of each 10° C. This has been generalized by VAN'T HOFF (8) into the rule that for every rise of 10° C. the rate of reaction is about doubled or trebled. It is also recognized that temperature influences the rate of physiological processes in plants and animals. It is known that an increase in light intensity increases photosynthesis, and a rise in temperature increases carbon dioxide assimilation, oxygen elimination and growth in plants and a fall in temperature decrease these processes with resulting dormancy. Some investigators (1, 5–7) claim that the temperature relations of these processes are such that they conform to the VAN'T HOFF rule for pure chemical reactions, while others (2–4) claim that they do not.

One of the earliest divisions of the animal kingdom into cold blooded, warm blooded, and hibernating animals, was made on the basis of temperature. It is known that oxygen absorption, carbon dioxide elimination, heat production, and hence oxidation is increased by a rise in temperature and decreased by a fall in temperature in cold blooded animals, resulting in a fall in body temperature and dormancy. Low temperature, on the other hand, increases oxygen absorption, carbon dioxide elimination, heat production, and hence oxidation in warm blooded animals, and is one of the factors responsible for the maintenance of the constant body temperature of these animals.

The object of this investigation was to determine the effect of various temperatures on the rate of sugar utilization by the plant *Spirogyra porticallis*, and by a cold blooded animal, the ordinary gold fish, in order to determine how closely the effect of temperature on sugar utilization coincided with its effect on pure chemical reactions.

The respiratory quotient is the index usually used to the amount of sugar metabolized, a rise in the quotient indicating an increase in sugar metabolism, and a fall, a decrease. In this investigation sugar utilization, as well as the effect of various temperatures on the rate of this utilization, was determined directly according to the following procedure.

Eight hundred cc. of 0.1 per cent dextrose solution was prepared and divided equally into eight beakers of 100 cc. each. Two gold fish of approximately the same size and with a combined weight of approximately 5 gm. were then introduced into each beaker. Air was bubbled through the sugar solutions to insure an adequate supply of oxygen to the fish. The beakers were then placed in water baths at 5°, 10°, 15°, 20°, 25°, and 30° C. respectively. A small amount of sugar solution was removed immediately from each of the beakers and sugar determinations were made according to the method of BENEDICT. At the end of the experiment, which lasted 24 hours, sugar determinations were made again. The results of the average of five series of such experiments are shown in fig. 1. It will be seen that the fish kept at 5° C. used 15 per cent of the sugar in 24 hours, and those kept at 10°, 15°, 20°, 25°, and 30° C. used 24, 30, 32, 35, and 46 per cent of the sugar respectively. By comparing these figures it will be seen that sugar utilization was decreased at the lower temperatures and increased at the higher. The effect on the sugar metabolism of the fish at 0° C. is not given in the chart, because it was found in most of the experiments that the fish died at this low temperature. It was not possible to keep the temperatures of the different baths absolutely constant as given in the chart; however, they were kept within less than 1° C. of the temperatures indicated.

The method of procedure in determining the effect of various temperatures on the rate of sugar utilization by *Spirogyra* was as follows. A considerable amount of *Spirogyra* was collected from a

nearby stream, brought to the laboratory, and the excess of water removed by gently squeezing with the hands. This material was then divided into smaller portions of 40 gm. each. Seven of these portions were introduced into 200 cc. of 0.1 per cent dextrose solutions in flat bottomed dishes 16 cm. in diameter. The dishes were then introduced into water baths at 0°, 5°, 10°, 15°, 20°, 25°, and

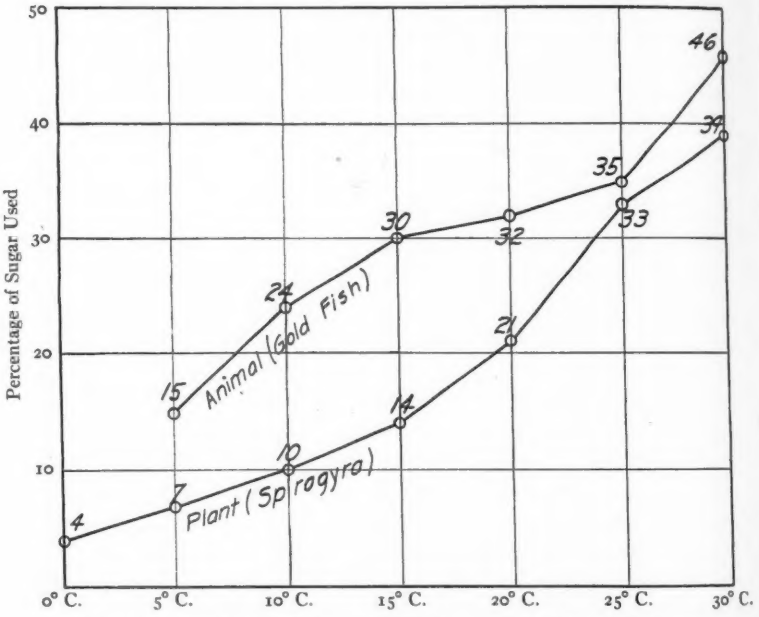

FIG. 1.—Curves showing that sugar utilization by gold fish and *Spirogyra* are decreased by low temperatures and increased by higher temperatures.

30° C. respectively and kept for 30 hours. A small quantity of sugar solution was removed immediately from each of the dishes and sugar determinations were made according to the method of BENEDICT. Sugar determinations were also made after 30 hours, at the end of the experiment. The results of the average of five series of experiments are shown in fig. 1. It will be seen that the *Spirogyra* kept at 0° C. for 30 hours used 4 per cent of the sugar; that kept at 5° C. used 7 per cent; and that kept at 10°, 15°, 20°, 25°, and 30° C. used

10, 14, 21, 33, and 39 per cent of the sugar respectively. By comparing these figures it may be seen that sugar utilization by *Spirogyra* was decreased at the lower temperatures and increased at the higher temperatures, just as was found to be the case with the gold fish.

A comparison of the two curves in fig. 1 brings out certain facts regarding sugar utilization by the animal and the plant. In the first place, it will be seen that the utilization of sugar by the animal was greater at all temperatures than that by the plant, although the weight of the animal used was only 5 gm. and the weight of the plant used was 40 gm. This is in keeping with the fact that metabolism in the animal is more intense than it is in the plant. In the second place, lowering of the temperature produced a greater decrease in sugar utilization in the plant than it did in the animal. At 25° C., for example, the amounts of sugar used by the plant and animal were almost the same, namely, 33 and 35 per cent respectively; while at a low temperature, 5° C., the amount of sugar used by the animal was more than twice as great as that used by the plant, namely 15 and 7 per cent respectively.

It was shown by VAN'T HOFF that if the logarithm of the reaction rate be plotted against the reciprocal of the absolute temperature at which the reaction takes place, a straight line is obtained in case of pure chemical reactions. The object was to plot curves, using the data given in fig. 1, for the effect of temperature on the rate of sugar utilization by the gold fish and *Spirogyra*, and to determine how closely they approach a straight line.[1] In fig. 2 the straight unbroken line is the theoretical curve obtained if the effect of temperature on the rate of sugar utilization by *Spirogyra* had been the same as it is on pure chemical reactions; while the broken line curve is constructed with the use of the data actually obtained from the effect of temperature on sugar utilization by *Spirogyra*. It may be seen that the broken line curve approaches very nearly the unbroken straight line curve, except at 0° C., and hence it may be concluded that the effect of temperature on the rate of sugar utilization by *Spirogyra* is the same as its effect on pure chemical reactions, except

[1] Thanks are expressed to Mr. D. F. BABCOCK of the Department of Physical Chemistry for the curves.

at this low temperature. The explanation that suggests itself for this exception is that the low temperature impaired the mechanism for normal physiological oxidation in the living plant. It is recognized that a relatively high temperature is required to bring about the oxidation of sugar in vitro, while in vivo this oxidation is brought

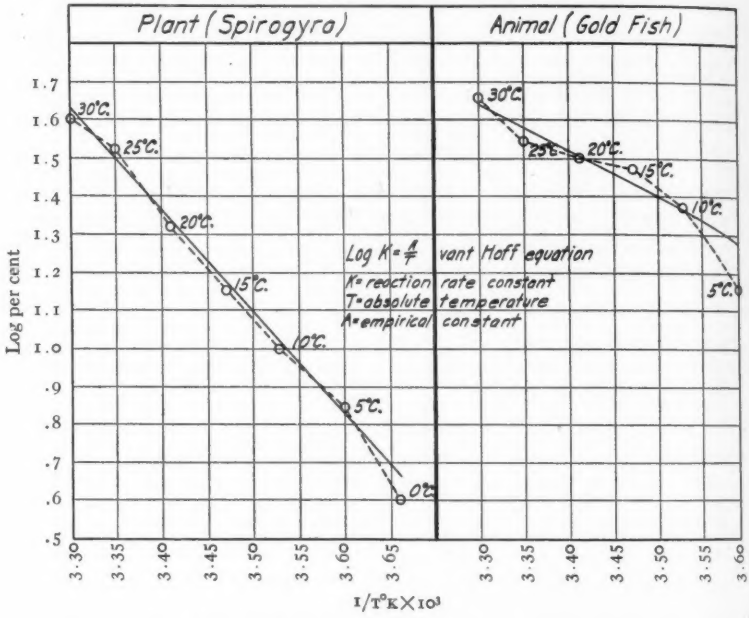

FIG. 2.—Chart showing that broken line curves plotted with use of experimental data on effect of temperature on rate of sugar utilization by *Spirogyra* and gold fish with use of VAN'T HOFF equation approach rather closely to straight line curve for pure chemical reactions.

about at the temperature of the living cell; hence there must be a mechanism whereby this oxidation can proceed at the temperature of the cell.

Fig. 2 also shows the curve plotted with the use of the VAN'T HOFF equation for pure chemical reactions, using the data obtained in fig. 1 on the effect of different temperatures on the rate of sugar utilization by the gold fish. It will be seen that the broken line curve,

which was constructed with the use of the data obtained with the gold fish, approximates roughly the straight unbroken line curve for pure chemical reactions, except at the temperature of 5° C. This approximation to a straight line, however, is not so close with the gold fish as it is with the *Spirogyra*. As with the *Spirogyra* so with the gold fish, the greatest deviation from the straight line curve, and hence from VAN'T HOFF'S law for pure chemical reactions, occurs at the lowest temperatures used. The possible reason for this deviation at the low temperature may be again that the low temperature of 5° C. impaired the normal physiological mechanism for the oxidative processes of the fish.

The following precautions were taken and checks made in the preceding experiments. Air was bubbled through sugar solutions for 30 hours without the presence of fish or *Spirogyra*, and it was found that this had practically no effect on the sugar solutions. It was found that the sugar was used only when the fish or the *Spirogyra* was present, and that the utilization ceased upon the removal of either. From these observations it was concluded that the fish and the *Spirogyra* were responsible for the utilization of the sugar observed in these experiments.

Summary

1. Raising and lowering the temperature produced an increase and decrease in sugar utilization by the plant *Spirogyra*, and by a cold blooded animal, the ordinary gold fish, just as it does in pure chemical reactions.

2. Lowering the temperature produced a greater decrease in sugar utilization by *Spirogyra* than by the gold fish.

3. The effect of temperature on the rate of sugar utilization by *Spirogyra* and the gold fish followed very closely VAN'T HOFF'S law for pure chemical reactions, except at very low temperatures. The explanation that suggests itself for this deviation from the law is that the normal physiological mechanism for sugar utilization in the living cells of the plant and animal is impaired by very low temperatures.

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OSMOTIC PRESSURE AND PH MEASUREMENTS ON CELL SAP OF PINUS PONDEROSA

FLOYD W. GAIL AND WM. H. CONE

(WITH TWO FIGURES)

Introduction

It has been found by the senior writer (1) that the osmotic pressure of expressed cell sap in some deciduous and non-deciduous plants reached a maximum during the winter months; that there was a decrease in the spring, and a gradual rise again with the coming of fall. It has also been shown by MEYER (3) that the sugar content of the expressed cell sap of the pitch pine increases during the autumn months, is relatively high during the winter, decreases in the spring, and is relatively low in the summer.

Since there is a relation between osmotic pressure and sugar content of expressed cell sap, and as sugars have a considerable influence on the pH value of solutions, it was thought desirable to measure the pH values of expressed cell sap of *Pinus ponderosa*, and determine whether any relation exists between the osmotic pressure and the pH values of the cell sap of this tree over an extended period of time.

Method

Branches were collected in the early morning from Cedar Mountain, which is about 9 miles distant from the University of Idaho campus. The needles were picked from the branches and dust particles removed as completely as possible by the use of soil sieves and cheesecloth. The leaves were then ground in a meat chopper, using the fine knife, and the cell sap expressed by the method previously evolved (1). Very little time elapsed between collecting the leaves and the osmotic pressure and pH determinations.

The freezing point depressions were determined by use of a Beckmann thermometer, and the osmotic pressure was computed by using the following formula:

12.05 (T−T′)=atmospheres of osmotic pressure
T′=freezing point of distilled water
T=freezing point of cell sap
12.05 is the ratio of the osmotic pressure, expressed in atmospheres, to the freezing point depression, in degrees centigrade, of a solution of a normal solute in water

The pH values were determined electrometrically in a Bailey hydrogen electrode, measured against a saturated calomel cell by means of a Leeds and Northrup potentiometer. The e.m.f. values so obtained were converted into the corresponding pH values from an unpublished table computed by Professor L. C. CADY of the Chemistry Department.

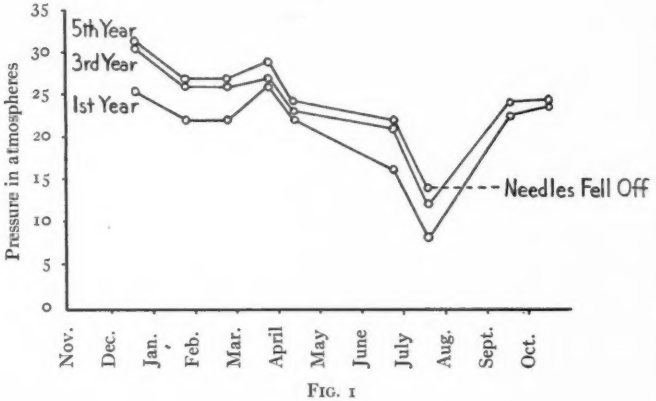

FIG. 1

Experimentation

Work was begun in November, 1925, and measurements were made once a month for a year, and at irregular intervals thereafter for another year. Determinations were made on the first, third, and fifth year needles. It was noted at once that the osmotic pressure and pH values were greater in the older needles, and this was found to be true without a single exception throughout the entire period of experimentation. The first osmotic pressure measurements were made December 28, at which time there was a maximum value for the year (fig. 1). A minimum pH (fig. 2) was obtained the latter part of January. From that time there was an increase in pH until

a maximum was reached in April. After this date the pH was practically constant until in July, with the exception of some variation in the first year needles. After July the pH values decreased gradually until November, when regular determinations ceased. A mini-

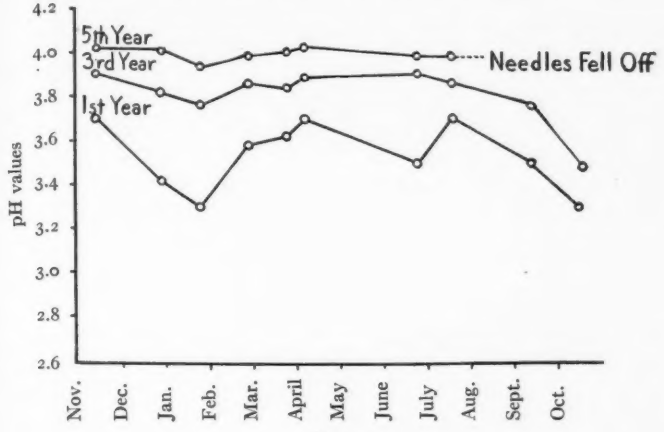

FIG. 2

mum osmotic pressure was reached during July when the cell sap still showed a high pH. The osmotic pressure increased from this time until the last measurements were made on these needles in

TABLE I

SUMMARY OF OSMOTIC PRESSURE VALUES

AGE OF NEEDLES	SPRING TIPS	FIRST YEAR	THIRD YEAR	FIFTH YEAR
Average value for one year.....	3.04*	22.15	24.13	24.46
Maximum value.....	.....	26.99	30.42	30.54
Minimum value for one year....	.....	7.23	11.57	12.05
Variation during one year.....	.....	19.76	18.85	18.49

* Average for spring tips represents average of monthly values from time of appearance in June until in September.

November. The fifth year needles fell during the month of August until it was impossible to collect enough for a determination (figs. 1, 2).

A consideration of the osmotic pressure values (table I) shows

an increase with the age of the needles and a decrease in the annual variation.

While there is not a direct relation between the osmotic pressures, as determined by freezing point depressions, and pH values, a summary (table II) shows an increase in pH with increase in the age of the needles and a decrease in the annual variation.

The lower osmotic pressures and the higher pH values of April, May, and June may be explained partially on the basis of rapid growth during these months. The carbohydrates and the other substances are used for growth of the new leaves and stems, resulting in a decrease of osmotic pressures.

TABLE II

SUMMARY OF pH VALUES

AGE OF NEEDLES	SPRING TIPS	FIRST YEAR	THIRD YEAR	FIFTH YEAR
Average values for one year	2.986*	3.525	3.635	3.991
Maximum value		3.702	3.888	4.142
Minimum value		3.195	3.466	3.753
Annual variation		.507	.422	.389

* Average for spring tips represents average of monthly values from time of appearance in June until in September.

It has been shown by LEWIS, MERRIAM, and MORAN (2) that the presence of sugars decreases the pH values. The substances including sugars made by photosynthesis, as well as stored materials being used for growth decreasing the sugar content, may in part account for the higher pH values during the months of April, May, and June. Growth largely ceases at this time, resulting in storage of starches and other substances. These may be changed to sugars, etc., resulting in increased osmotic pressures and decreased pH values during the winter months.

Just prior to the falling of the fifth year needles the pH was approximately 4.1. This was about the maximum value ever found in a great number of determinations covering a period of two years. Unpublished data on *Pseudotsuga taxifolia* also show 4.1 to be about the highest pH obtained in that species. This may possibly indicate that at such a pH value the enzyme action is inhibited to such an extent that life processes are no longer possible.

Summary

1. Osmotic pressure measurements made by means of freezing point depressions, and pH values determined electrometrically were made on first, third, and fifth year needles of *Pinus ponderosa*.

2. There is a high pH and low osmotic pressure during the period of growth, and a lower pH and higher osmotic pressure during the dormant period.

3. The pH and osmotic pressure of the cell sap increase with the age of the needles for any given time.

4. The fluctuation of osmotic pressures and pH values becomes less as the age of the needles increases.

5. The needles fell when the pH was approximately 4.1.

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[*Accepted for publication January 16, 1929*]

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MOTILE SPORES OF PEARSONIELLA

EARLE AUGUSTUS SPESSARD

(WITH TWENTY-SEVEN FIGURES)

The genus *Pearsoniella* was founded by FRITSCH and RICH[1] from material collected in South Africa. They state: "It is essentially characterized by its chloroplasts, which assume a form, as far as we are aware, not yet recorded among the algae, viz. that of a complete ring or girdle encircling the periphery of the cell." A letter recently received from the senior author expresses the opinion that the genus may have to be discarded after a complete renovation of the genus *Ulothrix* has been effected. PRINTZ[2] states: "Mir scheint es wahrscheinlicher, dasz sie nur eine Sektion von *Ulothrix* darstellt."

Since little is known about the method of reproduction in the genus, the following account may serve as an aid to the determination of its taxonomic position.

MATERIAL

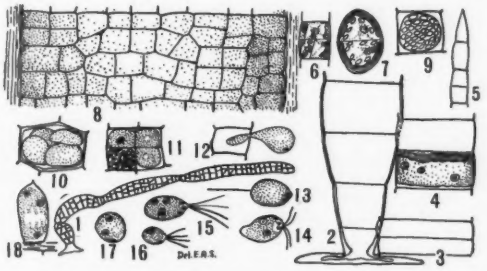
All the material on which this account is based was raised in the laboratory from a few young plants found in water gathered at a quiet spot in the Ouachita River at Arkadelphia, Arkansas, August, 1928. Within a few days the material fruited, as shown in figs. 24–27. This type of reproduction has been seen but once since that time.

On February 7 it was observed that these spores were motile and that one was produced in a cell. They germinated in the inclosure of the outer walls in case they did not escape. The cross walls were broken down, so that as many as eight appeared in a single inclosure. By October, some of the original filaments had enlarged to many-celled filaments (fig. 1). Such filaments, when placed in fresh cultures, disappeared, and young filaments were found on the sides of

[1] FRITSCH, F. E., and RICH, FLORENCE, Contribution to our knowledge of the freshwater algae of Africa. IV. Freshwater and subaerial algae from Natal. Trans. Roy. Soc. So. Africa. 11:314–317. 1924.

[2] PRINTZ, H., Ulotrichaceae, in ENGLER and PRANTL'S Die natürlichen Pflanzenfamilien. 3:157–165. 1927.

the container. Material from one-celled sporelings to filaments 200 μ in diameter have been grown within five months under laboratory conditions. Plants were observed in the living condition, by microphotographs, and after sectioning. All cytological details will have to be omitted until a thorough study of the material can be made.

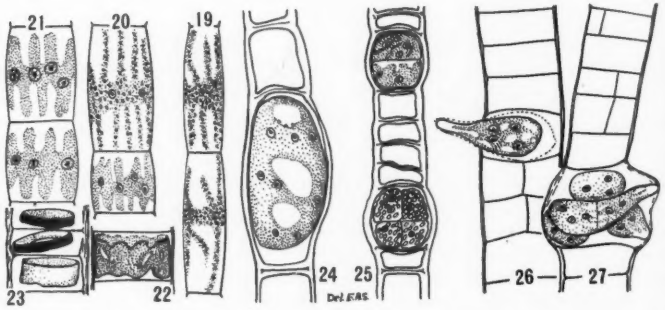


FIGS. 1–18.—Fig. 1, medium-sized filament of third phase of *Pearsoniella*. Figs. 2–5, portions of single filament from base to tip, at levels 5, 10, and 20 mm. from base; filament much narrowed at tip and widest below middle; lobed chromatophores shown in fig. 19 are present in apical or near apical cells of this filament. Figs. 6, 7, longitudinal and cross-section of filaments in second phase of longitudinal division. Figs. 9–16, stages in development and life of zoospores. Figs. 17, 18, stages in development of young sporeling; ×256 except fig. 1, which is ×55.

VEGETATIVE GROWTH

There are three comparatively distinct phases to the life history of *Pearsoniella* so far as observed. All of the stages appear to be vegetative. The first phase begins with the zoospore (figs. 12–16). This lasts during its motility, for half an hour to an hour. This short motile period is rather unusual; in *Ulothrix* the time is often 24 hours. The short period may be caused by the excessive light under the microscope, or possibly the heat. The second phase (figs. 2–5, 17–23) is the sporeling and elongation phase. The filament reaches a length of 2 cm. within 10 days, without any longitudinal division of the cells. Sometimes, however, longitudinal division begins in isolated cells before this length is reached. The third phase is initiated by longitudinal division of the cells. It continues until the filament attains a diameter of 200 μ. Zoospores are produced during the second and at the end of the third phase.

Mitotic figures have not been observed; consequently it is not safe to state precisely what is the nature of wall formation in the large filaments. It is not certain whether the filament is a hollow cylinder or a solid mass of cells. Sectioned material shows that there is a hollow space in the middle of some of the largest filaments of the third phase, nearing the production of zoospores. This point is being investigated. Fig. 7 shows a cross wall in a filament two cells wide. In fig. 6 is shown a longitudinal wall in a filament of the third stage at the beginning of longitudinal division. In some sections



FIGS. 19–27.—Figs. 19–23: band-shaped chromatophores in older and basal cells in figs. 22 and 23, as well as band-shaped and deeply lobed chromatophores characteristic of cells of second phase of vegetative growth; ×452. Figs. 24, 25: portion of two filaments showing stages in development of zoospores from young filament of third phase; possibly representing abnormality; ×256. Figs. 26, 27: germination and escape of zoosporelings in early stages of third phase; ×452.

there appears to be distinct apical activity, but these are isolated cases and cannot be used as evidence. From the outside appearance of the largest filaments, as well as the smallest, walls appear to show distinct relationship to the original divisions. In fig. 5 the third cell has divided without changing the contour of the mother cell In fig. 1, the many-celled filament of the third phase shows constrictions that suggest a zonal activity coordinate with the original cells of elongation like those shown in figs. 2–5. The filaments reach a length of at least 6 cm. and a width of 60 μ by the time the longitudinal walls become general.

The filaments of the third phase twist and bend about to form a

tangled mass 3 cm. in diameter. The size, of course, depends upon the number of threads present originally. From the culture records of sporelings observed, there is apparently no period of rest. One mass of filaments in the third phase was cut in half in November and it doubled its size by January 31. During this time two sets of sporelings were started from spores approximately 20 days apart. It appears that spores are produced periodically, and that the cells remaining in the large filament continue to vegetate.

Zoospore production

Zoospores have been observed to discharge on ten different occasions. On each occasion they were produced by filaments of the third phase. The first discharge occurred in material contaminated with other algae; the second discharge yielded but one zoospore; the third occurred at noon, and several hundred spores were observed from the moment of discharge until they settled down. Previous to these discharges sporelings of all stages had been seen.

Fig. 12 shows a zoospore emerging from the cell of a large filament. The passage out is always taken suddenly and singly. The mother cell shown in the drawing is semidiagrammatic, but living material shows that the passage is as shown in the figure. In some instances a long fine line (fig. 13) can be seen to hang on to the spore a few minutes after discharge. In one instance a small green spherical mass remained attached to this thread, and behind this a second irregular mass of protoplasm, starch, and chromatophores, four times the volume of the spore, was attached. The spore thus encumbered continued to drag its load, tandom fashion, from one side of the slide to the other.

Surface views (figs. 10, 11) leave some doubt as to the number of spores in each mother cell; sections no doubt will explain this point. In filaments as large as these it is impossible to observe whether spores discharging near one another, and very close to one another in time, really come out from one cell containing four or from one containing one. Fig. 8 shows the surface view of the filament from which the zoospores figured were discharged. The shading does not indicate the contents. In fig. 10 there is shown one cell with four zoospores. No membranes could be seen. Fig. 11 shows four cross

walls, but these may be separate from the wall proper at discharge. The tail-like affair shown in fig. 13 seems to be explained only on the assumption that a membranous sheath escapes with the zoospore.

Zoospore

The zoospore possesses four cilia slightly longer than the body. There are two contractile vacuoles situated in the clear region at the base of the cilia, and a short pointed beak from which the cilia arise. A red stigma is always present. The average diameter is 14 μ, and the average length 25 μ.

Fig. 16 shows a type of zoospore 9×15 μ. These spores have been seen to settle down and produce filaments. No instance of fertilization has yet been observed in this material. The exact nature of the spores shown in figs. 24–27 is not determined. They are motile, and in all healthy material observed only one to a cell was produced. In what respect the two types of spores differ is not yet determined.

From this brief account, *Pearsoniella* appears to have a much longer life history than has hitherto been supposed. The presence of the large zoospore-forming filaments will necessitate some slight changes in the original description. This the writer of course leaves to the judgment of the original authors, to whom material is being sent for inspection.

Summary

1. *Pearsoniella* is reported for Arkansas.

2. The mature vegetative stage is an immense, many-celled cylinder possessing a vegetative growth of at least two months.

3. The zoospores are produced at two stages in the life history, the early and late stages of the third phase, and last stages of the second phase.

4. Heterospory or heterogamy is suggested by the observation of unlike "zoospores."

5. No fertilization was observed.

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[*Accepted for publication February 6, 1929*]

BRIEFER ARTICLES

A HEAD OF SORGHUM WITH GREATLY PROLIFERATED SPIKELETS[1]

(WITH THREE FIGURES)

An unusual head of sorghum was found in a field of Blackhull kafir by D. L. ROSER, Burlington, Coffey County, Kansas, October 19, 1928. It was taken from a bundle at the time the crop was harvested and no other specimen of this kind was observed. The field was practically pure and nearly free of smut. The seed was reported to have been grown in Coffey County, and was obtained from the Burlington Feed and Seed Store. Blackhull kafir is an adapted and extensively grown variety in the vicinity of Burlington.

The panicle (fig. 1) was about the size and shape of a normal head. It was late in maturing, and contrary to the usual habit the spikelets at the tip of the panicle were last to reach full development.

Examination of a number of spikelets revealed the normal paired condition of the genus *Sorghum*. The upper spikelet was always small, about 3.5–4 mm. long, slender, villous on the pedicel at the juncture of the spikelet, and finely appressed hairy outside. It usually was made up of four scales (3–5), all of which were lanceolate, hyaline-margined, and sparingly appressed pubescent. No trace of stamens or pistils was discovered. The lower spikelet, however, was very curiously modified. Instead of the normally fertile condition, a series of scales extended upward from between the glumes (figs. 2, 3). A glance suggested a spikelet of an *Eragrostis*. No trace of grain or of grain-producing structures was found on any specimen examined. The spikelets varied in length from 3.5 to 10 mm. (usually about 9 mm. when fully expanded), and in width from 4 to 5.5 mm. (usually about 5.2 mm.). They were conspicuously villous at the juncture with the pedicel, in the same manner as the upper spikelets. When fresh, or later when soaked, these spikelets were the shape of a cone flattened at right angles to the scales, but when dried they were more or less oblong, except for the slight taper near the outer part and a prominent shallow groove running nearly the full length of the flattened sides.

[1] Contribution no. 181, Department of Agronomy and no. 292, Department of Botany and Plant Pathology, Kansas State Agricultural College, Manhattan, Kansas.

FIG. 1.—Unusual panicle of Blackhull kafir; about 1/3 natural size

The glumes were well developed, coriaceous, divergent, straw yellow, broadly ovate, and well rounded on the back, conspicuously hyaline and sparingly villous with projecting white hairs and slightly pubescent with appressed hairs. Above the glumes were about 38 (28–41) scales inserted closely, each ovate, or toward the tip of the spikelet ovate-lanceolate, well rounded on the back, bluntly or somewhat sharply pointed, but never

FIG. 2.—Cluster of spikelets showing unusual modification (divisions of scale below are millimeters).

awned, with 5 or 7 broad principal veins running obviously from near the tip. When magnified these appeared double, with two green stripes on either side of a white stripe. Between these veins there was usually an additional vein which stopped short of the others, but which was connected with them by veins running at right angles. In the soaked scale the vein network was observed to form a conspicuous pattern, which in the dry scales was obscured by the silky hairs that were particularly abundant toward the edge of the scale. Beyond the outermost veins was

a rather broad hyaline margin which infrequently was in part a deep red color. This color occurred more frequently on the lower or middle scales,

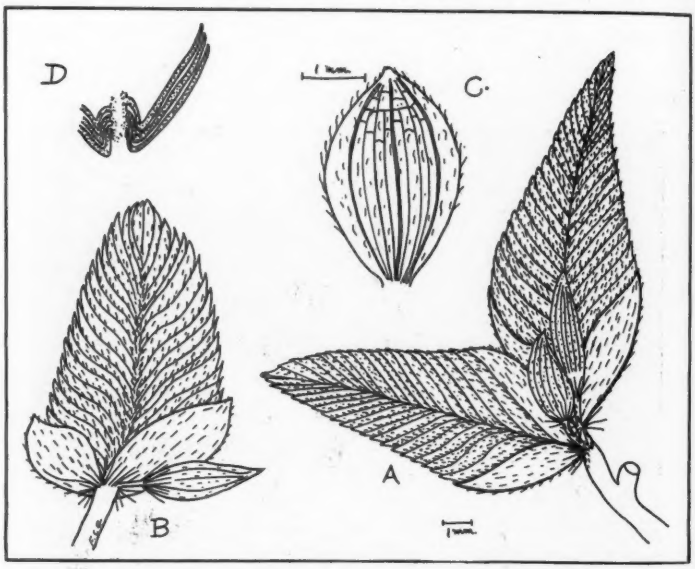

FIG. 3.—Diagram illustrating details of spikelets: *A*, fresh spikelets; *B*, spikelet thoroughly dried; *C*, single scale (lemma) showing peculiar venation; *D*, insertion of scales.

but was not sufficiently abundant to be a conspicuous point.—H. H. LAUDE and F. C. GATES, *Kansas State Agricultural College, Manhattan, Kan.*

[*Accepted for publication February 11, 1929*]

CURRENT LITERATURE

BOOK REVIEWS

Plant sociology

The trend of ecological thought in Europe has always differed somewhat from that which is at the time in vogue in America. It is peculiarly important, therefore, that American ecologists keep as close an understanding as possible of what is going on across the water. For this purpose the little volume by BRAUN-BLANQUET[1] is most admirable. His citation of literature is extensive, and extends right up to the date of publication. The very active Scandinavian and Russian ecologists are freely quoted, and much American research is reviewed. The book is a detailed summary of research methods, in fact, together with a critical evaluation of each proposal, and much sound philosophizing.

The introductory page points out the difference between studies of the individual organism and studies of communities. Choosing for this volume the second viewpoint, plant sociology is defined as the study of all the phenomena that have to do with the life of plants in social units. Section I discusses commensalism very briefly, and competition at length. Section II, the remainder of the book, presents in order the five principal problems of plant sociology: (1) organization or structure of communities; (2) synecology, the relation of communities to one another and to the rest of the environment; (3) syngenetics, the origin and decline of plant communities; (4) synchorology, the occurrence and distribution of communities; (5) classification of communities (systematics).

The first topic (organization) stands today at the focus of phytosociologic activities. The structure of the community "is the indispensable foundation for an unbiased treatment" of all the related problems. And it is this section that is most needed in American thought and practice. A difficulty arises in finding English equivalents for the terms Assoziation, Einzelbestand, Subassoziation, Fazies, Assoziationsfragment, Siedlung, Vegetationsfleck, Verein, Synusie, and Formation. It is indeed apparent that formation, association, society, as proposed by CLEMENTS and by LIVINGSTON and SHREVE, do not exactly correspond with the categories used by European botanists. It is to be hoped that a clear definition of terms may be decided upon at the forthcoming Cambridge conference.

The characteristics of a community are considered under the following heads:

1. Analytic characteristics.—

(a) Quantitative: number of individuals (abundance) and density; cov-

[1] BRAUN-BLANQUET, J., Pflanzensoziologie. pp. x+330. *figs. 168*. Berlin: J. Springer. 1928.

ergrade, volume and weight (dominance); grouping (sociability) and distribution; frequence.

(b) Qualitative: layering; vitality (health); periodicity.

2. Synthetic characteristics.—

(a) Constancy.

(b) Fidelity.

These concepts are clearly defined, and procedure is given for their determination. The result is shown by an example. Such procedure permits accurate comparison of data taken in any kind of vegetation in any part of the world.

Synecology treats in the usual way of heat, light, water, and wind. The section on soil is much more complete than in any other such text known to the reviewer, and is full of excellent data and constructive criticism. Life-forms are treated in the latter part of this section.

Syngenetics has to do with the up-building of communities, and includes the study of succession. About twelve pages suffice to outline this topic. The chapter ends with recent geological stages and pollen analysis.

Synchorology deals with the geographic extent and distribution of communities, both in breadth and altitude. Zonation is discussed here. Pioneer and relict communities are considered. The surface of the earth from the standpoint of vegetation is divided under the headings Region, Provinz, Sektor, Bezirk, District, Unter-district (Gau).

The classification of communities, "like every natural classification, presupposes the most exact knowledge of all parts of the problem. It follows from the nature of the case that this portion of the science of communities, lacking as yet the broad foundations that are necessary, is at present the least developed." Proposed systems of classification are cited and criticized. The last two pages offer a classification of communities based upon their sociological progression from simpler to more complex structures. Without attempting to be syngenetic or successional in arrangement, the series begins with the indefinite floating vegetation of air and ocean with few species (bacteria, algae, fungi), and culminates in the many-layered forest community with a multiplicity of species. It has the merit of simplicity, and it seems to provide a place for every kind of natural grouping of plants.

BRAUN-BLANQUET has given us in this work an ecological guidebook of first importance. The volume is crammed with ideas, methods, and results. The author has a fondness for inverted sentences which are somewhat difficult to the Anglo-Saxon mind; but his style is forceful, and his treatment analytic and fair. His examples bring out every point and process with clearness. The halftone illustrations of natural vegetation are really beautiful, and to the point. The book seems to be without a mechanical flaw.—H. S. CONARD.

Plant ecology

More than thirty years ago two centers of ecological study and teaching developed simultaneously in America, at the Universities of Chicago and Ne-

braska, and both schools have since contributed largely to the development of this branch of plant science, agreeing in placing great emphasis on the dynamics of vegetation as expressed in plant succession. Both schools have been more interested in the investigation of the problems of vegetation than in the production of textbooks.

As the years have passed, however, and as some order has emerged from the intricacies of the relations between the plant, or the plant community, and its environment, the need of at least one comprehensive text on plant ecology has been felt. This demand has now been supplied from one of the original centers.[2] It seems particularly fitting that this book should have been written by Nebraska's pioneer ecologist, CLEMENTS, and one of his students, WEAVER, the latter for many years a successful investigator and teacher.

Perhaps the first impression gained by glancing through the 500 pages of this volume is the diversity of the topics discussed. Nor is this diversity in topics surprising when the complexity of the plant environment is recognized, and the varied applications now being made of the results of ecological investigations appreciated. An effort has been made, and a very successful effort, to discuss all these topics in a somewhat exhaustive manner. Among the portions of the text that seem to the reviewer most satisfactory, are the chapters or sections dealing with plant succession, competition and invasion, methods of studying vegetation, structure and ecology of roots, measurement and effects of evaporation and temperature, plant indicators, and the climax formations of North America. A map, which is remarkable for its simplicity, gives the limits of these formations. The units of vegetation, soil formation and soil structure, and the adaptations of plant structures seem to be less satisfactorily discussed.

With the great advances made in soil science during the past two decades, it is quite impossible to discuss the subject adequately within the limits of a dozen pages, nor is it surprising that an attempt to do so is rather unsatisfactory. It is noted, for example, that the difference between the hygroscopic coefficient and the wilting coefficient of soils is not clearly expressed. It is made clear that each is a function of the texture of the soil as related to its water content, but of each it is said that it represents the amount of water remaining in the soil when permanent wilting of plants occurs, and yet the wilting coefficient is nearly twice as great as the hygroscopic coefficient.

The units of vegetation are still burdened by an excessive terminology, a fault that seems evident in other portions of the book.

The rapid advance now being made in certain branches of plant ecology is nowhere more clearly shown than by a comparison of the chapter on leaf structure or "adaptation to water" with a corresponding chapter in MAXIMOV'S[3]

[2] WEAVER, J. E., and CLEMENTS, F. E., Plant ecology. 8vo. pp. xx+520. *figs. 262; colored map.* New York: McGraw-Hill Book Co. 1929. $5.

[3] MAXIMOV, N. A., The plant in relation to water. London: George Allen & Unwin Ltd. 1929.

book, which appeared only a few weeks later than the American text. We believe that WEAVER and CLEMENTS, together with most ecologists, will be convinced by the experimental data presented by MAXIMOV that such xeromorphic structures as greater thickness of leaves, more palisade tissue, more conductive and mechanical tissue, smaller epidermal cells (that are more highly cutinized and are accompanied by smaller but more numerous stomata), result in an increase and not in a decrease in transpiration. It is true, however, that leaves with such xeromorphic structures are more drought resistant in spite of their higher transpiration.

Notwithstanding such slight and largely unavoidable deficiencies, and certain minor errors which it would be invidious to enumerate, the authors are to be congratulated upon the successful outcome of their efforts. They have presented a multitude of data in a logical and well organized manner. Their discussion is broad and convincing; their citations show a thorough acquaintance with the latest as well as with the older literature. There is an occasional leaning toward teleological interpretation, but on the whole there is continued evidence of scientific accuracy. At times, however, the authors speak with a definiteness and conviction that most students of ecology find it impossible to share.

This volume may not commend itself to those who wish to teach a textbook; but for teachers and students really interested in science it will be a standard text for many years. While its organization may not exactly fit the needs of any particular class, it abounds in excellent suggestions for laboratory and field exercises, and will be found an indispensable aid in interpreting the innumerable phenomena of vegetation. The well chosen illustrations and the extensive bibliography also help in making it by far the best and most comprehensive textbook on the subject that has yet appeared.

The printers and publishers have done their share in producing a book which it will be difficult to excel.—G. D. FULLER.

Plant rusts

The fields of mycology and phytopathology are so vast that one person cannot keep abreast of all their aspects. Consequently, the volume on the plant rusts by ARTHUR[4] and his students and associates is a most welcome and timely addition to mycological and phytopathological literature, by a group of competent specialists. It is hoped that their example will be followed, and that separate comprehensive treatises on other biologically interesting groups of fungi, such as the Ustilaginales, for example, will appear in the near future. Such volumes should summarize what is accepted as established fact and established concept, point out what is problematical, and suggest working hypotheses to fill in the gaps and to stimulate new investigations. The volume under discussion in great measure lives up to these demands. It is characterized

[4] ARTHUR, J. C., in collaboration with KERN, F. D., ORTON, C. R., FROMME, F. D., JACKSON, H. S., MAINS, E. B., and BISBY, G. R., The plant rusts (Uredinales). pp. v+446. *figs. 186.* John Wiley and Sons. 1929.

by comprehensiveness, clarity of expression, and an interesting, easy style. It contains chapters on: general nature of the rusts; historical review; development and classification; cytology and morphology; dissemination and geographic distribution; physiology; specialization; teratology and pathology; ecological considerations; methods of investigation; and a comprehensive bibliography. The value of the volume would have been greatly enhanced if a key and list of known rusts had been included.

It is stated (page 2) that the terms gametophytic or haploid phase, and sporophytic or diploid phase are not strictly applicable to the rusts. Certainly the terms gametophytic and sporophytic are not at all applicable, especially the former; but it does not follow, therefore, that the terms haplophase and diplophase are not applicable. In fact, their use would make for precision and clarity. Really to express the situation one should state that the life cycle of a "typical" rust is composed of three phases, the haplophase, the dicaryophase, and the diplophase. It is unfortunate that the authors have used the concepts and terms gametophytic and sporophytic as the framework of a great deal of their presentation.

At the beginning of chapter III the fundamental features of rusts are stated quite canonically. The following statement occurs: "the vegetative body is macrocyclic (long cycle), consisting of two unlike and discontinuous generations." Later in a discussion which justly stresses the importance of the mycelium it is stated "the aecia are initiated by the gametophytic mycelium." It may develop that these statements really represent the facts, when rust pustules are reinvestigated in the light of recent reports by CRAIGIE, HANNA, and ALLEN, but one is justified in hazarding the guess that more cases will be found of more or less extensive continuous development of dicaryomycelium from one or two haplomycelia, and of organization of aecia by either the haplomycelium and the dicaryomycelium, or by the dicaryomycelium alone. Of course, one can ask, When is an aecium an aecium? Should differentiation of the structures described by CRAIGIE, HANNA, and ALLEN in monosporidial pustules be considered as initiation of aecia? It is of interest that Miss ALLEN, in referring to these structures, uses the guarded phrase "structures resembling aecia." Although many guarded statements occur relative to sexuality, a definite stand is taken in chapter III for sexuality on the insecure ground that fusion or union occurs between hyphae, and eventually between nuclei.

One has the feeling that much needless effort was spent in harmonizing divergent views of the various contributors. (It is stated in the preface that "there remain here and there occasional statements which are not endorsed by one or more of the several authors.") Possibly the volume would have been even more stimulating than it is, if at the cost of no more than artificial harmony and unity, each contributor had been given free rein to express his own ideas and assume full responsibility for signed chapters.

It is a timely volume, admirably summarizing the present state of knowledge relative to rusts. It comes at a time when genetic analysis of fungi is in

the air and a new theoretical and experimental approach to the problem of specialization and of physiological races and forms is imminent, in the light of CRAIGIE's discovery of heterothallism in the Uredinales, of GOLDSCHMIDT's highly suggestive genetic analysis of specialization in *Ustilago violacea*, and of DODGE's interesting study of hybridization with *Neurospora*.—G. K. K. LINK.

Insects

Three volumes on insects have appeared which are of general biological interest, and lend themselves to use by anyone interested in plants and their enemies. The volume by METCALF and FLINT admirably covers its chosen field.[5]

The first chapters are devoted to a general consideration of insects, such as their importance to man and domestic animals, and their structures and life histories. These are followed by a presentation of the orders of insects, and a discussion of control measures. The remainder of the major portion of the book is devoted to a special part in which crops form the basis of discussion of insects which parasitize them. The volume is a signal contribution in the field of entomology.

The volume by GRAHAM,[6] although it is independent and is built along different lines, forms a companion book to the former, in that it takes up forest diseases, which are not considered by METCALF and FLINT. Because of the great importance of insects in the forest and in relation to timber decay by fungi, this volume will be welcomed by phytopathologists. The first four chapters are devoted to general biologic considerations relative to insects. Chapters VII–XIX are devoted to general control measures; chapters X–XV take up insects on the basis of the tree tissues attacked by them; and chapters XVI and XVII discuss the parasites of forest insects.

The volume by WARDLE[7] is evidence that entomology is ceasing to be a congeries of isolated facts about individual insects, with a casual glance at the host they infest, and is striving to be a real science. The first part of this interesting volume, which is permeated by a theoretical atmosphere, is devoted to a consideration of general problems such as host resistance, theory of insecticides, and cultural influences. The second part concerns itself with area problems, in which in addition to general theoretical considerations, the material for each major geographic area is presented so that the non-resident may form some idea of the problems which occur in that area. This is of great use in connection with quarantine problems. There is an excellent bibliography.—G. K. K. LINK.

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[5] METCALF, C. L., and FLINT, W. P., Destructive and useful insects. pp. xii+918. *figs. 561*. New York: McGraw-Hill Book Co. 1928.

[6] GRAHAM, S. A., Principles of forest entomology. pp. xiv+339. *figs. 149*. New York: McGraw-Hill Book Co. 1929.

[7] WARDLE, R. A., The problems of applied entomology. pp. xii+587. *figs. 31*. New York: McGraw-Hill Book Co. 1929.

Flora photographica

A very significant and comprehensive study of the vegetation of the world has just been initiated in a volume[8] of which Dr. HUGO ILTIS of Brunn is the editor. This book has for its main object the publishing of actual photographic prints, depicting the chief vegetative features of the entire world, country by country. The first volume to appear is volume II, which deals with the floral province of the European "Mittelgebirge." In the preface it is noted that this work is designed not only for botanists, but also for geologists, agriculturists, and friends of the plant world. The photographs are to be of uniform size, 9×12 cm., and it is hoped that this work will prove a notable complement to herbaria and floras. The publishing of photographic prints rather than the reproductions from these prints is highly advantageous, because none of the details of the picture are lost. It is proposed to publish two or three numbers annually, either in centuries or half centuries. In each volume a certain floristic area will be dealt with, on the basis of ENGLER'S classification. The work is published in three editions, German, French, and English. The volume under review was translated by Mr. W. C. WORSDELL. It has a complete index of plant names. In this first century the photographs are grouped in twenty-five different formations, some of which are the *Pinus silvestris* forest, birch forest, spruce forest, mixed mountain forest, beech forest, hornbeam forest, lowland and riverside forest, forest clearing, the copse and forest-edge, heath, rock, dry mountain-meadow, valley and riverside meadow, and moorland meadow. If this first volume to appear is any indication of what is to follow, we cannot have the later numbers too soon. In the opinion of the reviewer, no such great and concise treatise of the vegetation of the world, area by area, has been presented in a form so easily understandable.—H. C. COWLES.

NOTES FOR STUDENTS

Anthocyanins.—The anthocyanins have long been of interest to botanists as natural indicator pigments. Recently FEAR and NIERENSTEIN[9] have pointed out that the color reactions of these compounds should be examined in solutions of definite pH, if such studies are to be valuable in comparison and characterization of the various anthocyanins and their derivatives. It is now claimed by ROBERTSON and ROBINSON[10] that examination of the reactions of the anthocyan-

[8] ILTIS, H., and SCHULZ, B., Flora Photographica. II. Floral province of the European "Mittelgebirge" I. Transl. by W. C. WORSDELL. pp. 50. *pls.* 100. Brunn: Rudolf M. Rohrer. 1928.

[9] FEAR, C. M., and NIERENSTEIN, M., The colour variations of cyanidin chloride and 3:5:7:3′:4′–pentahydroxyflavylium chloride as related to acidity and alkalinity. Biochem. Jour. 22:615–616. 1928.

[10] ROBERTSON, A., and ROBINSON, R., Note on the characterization of the anthocyanins and anthocyanidins by means of their colour reactions in alkaline solutions. Biochem. Jour. 23:35–40. 1929.

idins in a range of buffered solutions is by far the most reliable method for such comparisons, even of greater usefulness than the study of absorption spectra, for some different anthocyanidins (as peonidin and syringidin), as demonstrated by SCHOU,[11] show identical absorption sepectra, but can easily be differentiated by their reactions in the presence of buffered solutions. At the same time, the buffer method of examination reveals such phenomena as pseudo-base formation, color-base precipitation, and the ease of oxidation of the compounds.

ROBERTSON and ROBINSON do not agree with FEAR and NIERENSTEIN that cyanidin chloride differs from synthetic 3:5:7:3′:4′-pentahydroxyflavylium chloride, but emphatically insist that the pure synthetic compound, prepared from its benzol derivative by hydrolysis, exhibits no differences, even to the minutest details, from the natural cyanidin chloride. Claiming that the structural constitution of the carbon-oxygen skeleton of cyanidin chloride is as firmly established as that of any other compound, they reject the attempt of MALKIN and NIERENSTEIN[12] to modify the cyanidin formula to reconcile some earlier and conflicting studies in catechin chemistry.

The series of buffers used by ROBERTSON and ROBINSON were made from phenyl-acetic acid, boric acid, and KH_2PO_4, 0.02 gm. each, with additions of different amounts of 0.2 N NaOH, dissolved in water and made up to 1000 cc. The range in seventeen steps was from pH 3.2 to approximately pH 13. The reactions of various anthocyanidin chlorides are described after standing 24 hours in the buffers. They include the chlorides, apigeninidin, pelargonidin, cyanidin, peonidin, and malvidin. The reactions of the chlorides of two anthocyanins, cyanin and malvin, are also described. The changes with changing pH and with time are distinctive, and the method should prove valuable in grouping the many naturally occurring but yet unstudied anthocyanins.—C. A. SHULL.

Taxonomic note.—Another posthumous part of the late J. H. MAIDEN'S monumental revision of *Eucalyptus*[13] is at hand. No new species and in fact only two new varieties are described. There is appended a digest of opinions from various sources as to "the species question." These bear upon such topics as: what is a species, variety or species, inequality of species values, no fixed line of demarcation between species, JORDAN'S species, classical case of splitting, application of zoological tests to a botanical species, and variation in the genus. They are followed by a section on the struggle for taxonomic definiteness, in which are given discussions of the ideal of the type, how to designate the type, model descriptions, and labels and schedules; and finally, a list of pertinent aphorisms taken from DARWIN, HUXLEY, and others.—E. E. SHERFF.

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[11] SCHOU, S. A., Über die Lichtabsorption einiger Anthocyanidine. Helv. Chim. Act. 10:907–915. 1927.

[12] MALKIN, T., and NIERENSTEIN, M., Zur Kenntnis des Cyanidins. I. Vergleichende Untersuchungen über Cyanidinchlorid und 3.5.7.3′.4′-Pentaoxy-flavyliumchlorid. Ber. Deutsch. Chem. Ges. 61:791–799. 1928.

[13] MAIDEN, J. H., A critical revision of the genus *Eucalyptus*. 7:405–450. 1928.

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Classified entries will be found under Contributors and Reviews. New names and names of new genera, species, and varieties are printed in **bold-face** type; synonyms in *italics*.

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